Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Discovery of Spirofused Piperazine and Diazepane Amides as Selective Histamine-3 Antagonists with in Vivo Efficacy in a Mouse Model of Cognition

Dean G. Brown,*[,†,∥] Peter R. Bernstein,[†] Andrew Griffin,[‡] Steve Wesolowski,[†] Denis Labrecque,[‡] Maxime C. Tremblay,[‡] Mark Sylvester,[†,∥] Russell Mauger,[†] Phillip D. Edwards,[†] Scott R. Throner,[†,∥] James J. Folmer,[†] Joseph Cacciola,[†] Clay Scott,[†,∥] Lois A. Lazor,[†] Mehrnaz Pourashraf,[†] Vijayaratnam Santhakumar,[†] William M. Potts,[†] Simon Sydserff,[†] Pascall Giguère,[§] Carine Lévesque,[§] Mohammed Dasser,[§] and Thierry Groblewski[‡,⊥]

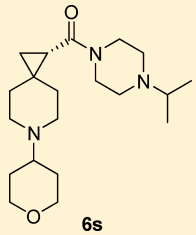
[†]AstraZeneca CNS Discovery Research, 1800 Concord Pike, Wilmington, Delaware 19850, United States

[‡]AstraZeneca Montréal, 7171 Frédérick-Banting, Montréal, Québec H4S 1Z9, Canada

[§]OmegaChem, 480 Rue Perreault, Saint-Romuald, Québec G6W 7 V6, Canada

Supporting Information

ABSTRACT: A new series of potent and selective histamine-3 receptor (H_3R) antagonists was identified on the basis of an azaspiro[2.5]octane carboxamide scaffold. Many scaffold modifications were largely tolerated, resulting in nanomolar-potent compounds in the H_3R functional assay. Exemplar compound **6s** demonstrated a selective profile against a panel of 144 secondary pharmacological receptors, with activity at only $\sigma 2$ (62% at 10 μM). Compound **6s** demonstrated free-plasma exposures above the IC_{50} (~50×) with a brain-to-plasma ratio of ~3 following intravenous dosing in mice. At three doses tested in the mouse novel object recognition model (1, 3, and 10 mg/kg s.c.), **6s** demonstrated a statistically significant response compared with the control group. This series represents a new scaffold of H_3 receptor antagonists that demonstrates in vivo exposure and efficacy in an animal model of cognition.



6s

hH_3 GTPγS IC_{50} = 8.3 nM
hERG > 33 μM
Phospholipidosis in vitro EC_{50} >300μM
Secondary pharmacol: 143/144 >10 μM
%F = 99
Mouse novel object recognition < 1 mg/kg (s.c)

INTRODUCTION

The histamine-3 receptor (H_3R) is an autoreceptor that operates presynaptically in histaminergic neurons.[1] Evidence suggests that selective blockade of H_3R results in increased levels not only of histamine but also of noradrenaline, acetylcholine, and dopamine.[2] As such, H_3R antagonists have been the focus of many recent drug discovery efforts, with interests spanning sleep disorders, cognition, attention deficit hyperactivity disorder (ADHD), osteoarthritis, Tourette syndrome, neuropathic pain, and metabolic disorders.[3−11] Over the years, selective antagonists at this receptor evolved from analogues of the endogenous ligand histamine into more advanced ligands that arguably are structurally distinct from histamine because they lack the imidazole chemotype.[12] Publications continue to emerge with a diversity of core scaffolds capable of inhibiting H_3 receptors.[13] Cardiovascular safety has proven to be an area of recurring concern, potentially because of off-target effects such as those arising from *human ether-à-go-go-related gene* (hERG) potassium channel binding.[14]

In the course of this project, we discovered a new class of selective H_3R antagonists. Of interest to us in this class of molecules was the lack of an aromatic ring that is present in most structures within the published H_3 literature. We hypothesized that the lack of aromaticity in the pharmacophore may reduce off-target issues.[15] The challenges of safety of H_3 antagonists have been recently discussed in a review by Plancher,[11] in particular, hERG, phospholipidosis (PLD), and mutagenicity, which can be mitigated in certain structural classes but still remain an issue with this type of pharmacophore. Because hERG tends to favor large hydrophobic groups often satisfied by hydrophobic aromatic elements,[16] this series, which exemplified the exclusion or reduction of aromatic rings, offered the opportunity to test our hypothesis. Our investigation began with 6-azaspiro[2.5]octane carboxamides (spirocyclopropyl core) and then expanded to core modifications such as 7-azaspiro[3.5]nonane carboxamides (spirocyclobutyl core) as well as five- and six-membered ring spirofused analogues. A number of molecules made in this effort possessed two basic groups, which is a feature present in the pharmacophore of other H_3 antagonists such as the natural products Aplysame-1[17] and Conessine[18a−c] (Figure 1) as well as the two synthetic examples from JNJ, namely, JNJ-5207852 and Bavisant (JNJ-31001074).[13e,f,19,20] Both the piperazine and diazepane scaffolds have been useful pharmacophoric elements in the field, with a wide amount of structural diversity of

Received: September 24, 2013

   dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

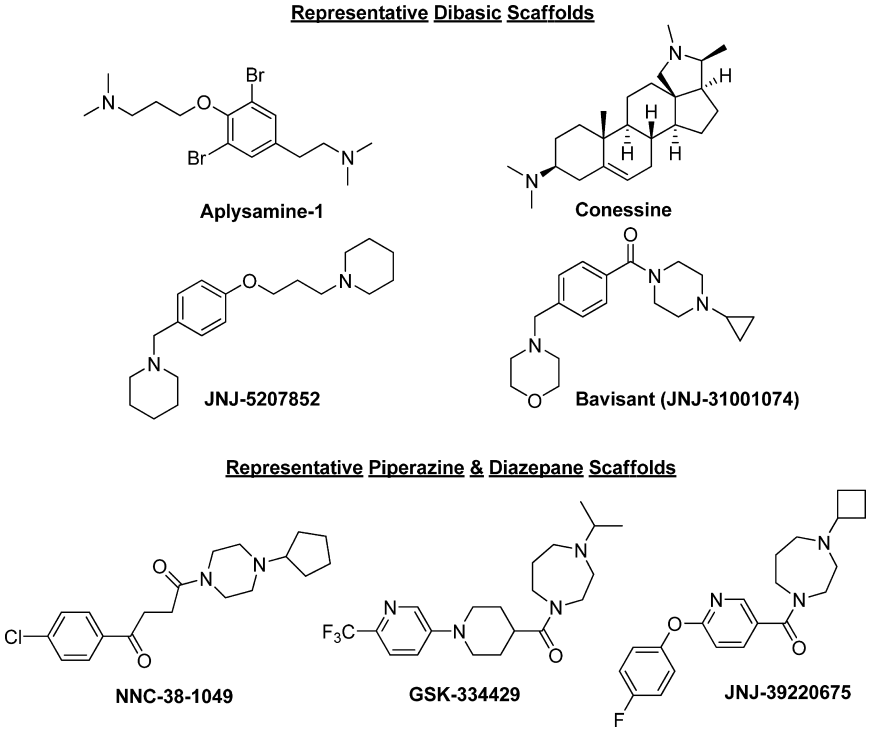


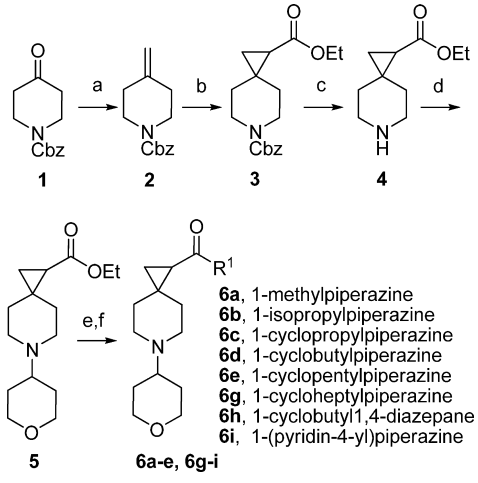
Figure 1. Representative H_3 scaffolds with dibasic, piperazine, and diazepane scaffolds.

attachments to these cores. Three examples of advanced compounds in the field with these elements are shown in Figure 1 (NNC-38-1049,[21] GSK 334429, and JNJ 39220675[22,23]). We report herein the structure−activity relationships (SAR), in vitro drug metabolism pharmacokinetics (DMPK), and in vivo assessment of example molecules from this effort.

CHEMISTRY

The synthesis of the basic core 6-azaspiro[2.5]octane carboxamide scaffold is shown in Scheme 1. Intermediate exocyclic olefin **2** was generated by Wittig olefination of Cbz-protected piperidin-4-one **1**. The spirocycle was then introduced by reaction of CuCN and ethyldiazoacetate to give ester **3**. Deprotection of the Cbz group with catalytic hydrogenation gave free amine **4**, which was subsequently alkylated under reductive amination conditions to give intermediate ester **5**. To finish the sequence, the ester was cleaved, and the carboxamide was introduced by coupling to the appropriate piperazine to give final products **6a−e** and **6g−i**.[24]

Scheme 1[a]



[a]Reagents and conditions: (a) $Ph_3PCH_3^+Br^-$, *n*-BuLi, THF, 54%; (b) CuCN, ethyldiazoacetate, CH_2Cl_2, 45%; (c) EtOH, Pd/C, H_2, 44%; (d) 1,2-DCE, dihydro-2*H*-pyran-4-(3*H*)-one, $Na(OAc)_3BH$, rt, 64%; (e) LiOH, MeOH, quan.; (f) HBTU, DMF, DIPEA, amine, 34−80%.

An alternative method was employed to add versatility for incorporating diversity elements to the route in Scheme 1, which is illustrated in Scheme 2. Intermediate **3** from Scheme 1 was hydrolyzed to give intermediate acid **7**, which was then coupled with Boc-protected piperazine to provide **8**. The Cbz group on the piperidine nitrogen was then removed under catalytic hydrogenation to provide **9** and subsequently reacted with a ketone under reducing conditions to provide **10**. At this point, the piperazine was deprotected to provide free amine **11**, which was subjected to a second reductive amination to give final compound **6f**.

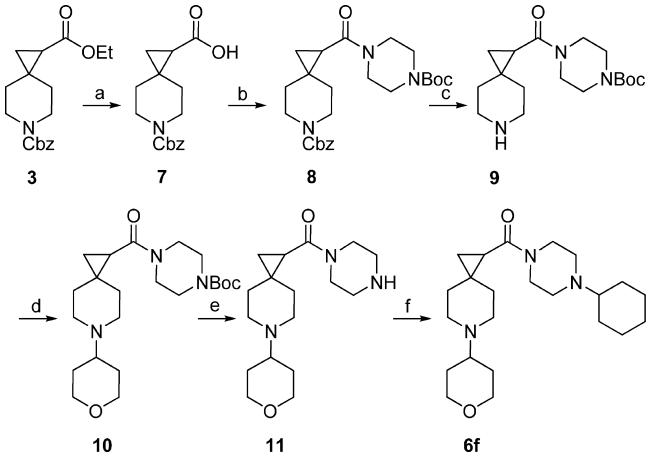
Entry into analogues wherein the two N-alkyl groups were reversed is outlined in Scheme 3. Intermediate **7** was coupled with 1-(tetrahydro-2*H*-pyran-4-yl)piperazine to provide **12**, which in turn underwent reductive amination, providing **6j**.

A series of spiropiperidine analogues were made in a similar fashion where the piperazine ring was held constant with a cyclohexyl substituent. The synthesis of these analogues is provided in Scheme 4. Intermediate **7** was acylated with 1-cyclohexylpiperazine to provide **13** and deprotected to give **6k**. This intermediate was acylated, alkylated, or aminated under Buchwald−Hartwig conditions[25] to provide title compounds **6l−q**.

Optically pure material was accessed through the route illustrated in Scheme 5. Racemic Cbz-protected ester **3** was separated by chiral supercritical fluid chromatography (SFC) to

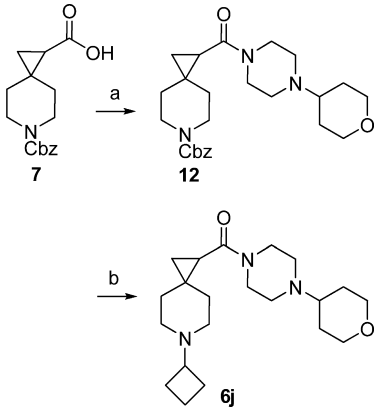
dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2[a]



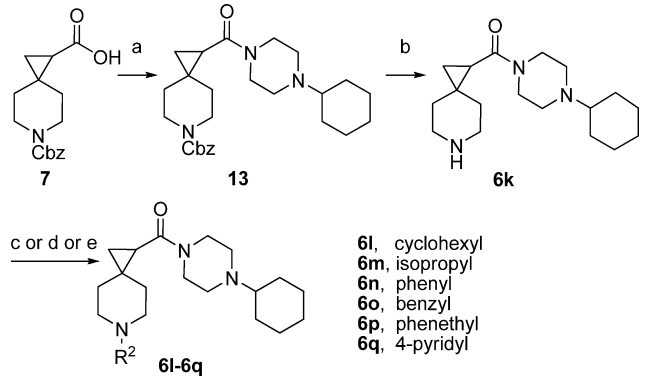
[a]Reagents and conditions: (a) LiOH, THF, 94%; (b) HBTU, DIPEA, *tert*-butyl piperazine-1-carboxylate, 69%; (c) EtOH, Pd/C, H_2, AcOH (cat.), 99%; (d) EtOH, $NaBH_3CN$, AcOH (cat.), dihydro-2*H*-pyran-4-(3*H*)-one; (e) CH_2Cl_2, TFA, 99%; (f) CH_2Cl_2, cyclohexanone, PS-$CNBH_4$, 66%.

Scheme 3[a]



[a]Reagents and conditions: (a) HBTU, 1-(tetrahydro-2*H*-pyran-4-yl)piperazine dihydrochloride, 11%; (b) Pd/C, H_2, MeOH, cyclobutanone, 46%.

Scheme 4[a]



[a]Reagents and conditions: (a) DMF, HBTU, DIPEA, 1-cyclohexylpiperazine, 70%; (b) MeOH, Pd/C, H_2, rt, 95%; (c) 4-Cl-pyridine, K_2CO_3, DMSO, 26%; (d) PhMe, Li(HMDS), PhBr, $PdCl_2[P(o\text{-tol})_3]_2$, 12%; (e) 1,2-DCE, R_2-CHO, $Na(OAc)_3BH$, rt, 30–60%.

provide pure stereoisomers **3a** and **3b**. The Cbz groups were removed by hydrogenolysis to provide intermediate amines **4a** and **4b**, which were assigned to their absolute relative configurations using computed and observed vibrational circular dichroism (VCD) spectra (Figure 5).[26]

Cbz-protected intermediates **3a** and **3b** were hydrolyzed with aqueous sodium hydroxide to provide (*R*) and (*S*) stereoisomers **7a** and **7b**, respectively. The optically pure acids were coupled to 1-isopropylpiperazine to provide Cbz-protected piperazines **14a** and **14b**. Deprotection and reductive amination were conducted in the same reaction to provide final compounds **6r** and **6s**.

The synthesis of an analogue in which the spiropiperidine basic nitrogen was removed and replaced with a spirocyclohexane is outlined in Scheme 6. Starting with ketone **15**, Wittig olefination was performed to provide ester **16** with 72% yield. Cyclopropanation was then achieved using dimethylsulfonium ylide under basic conditions to provide **17**. Subsequent hydrolysis and amide bond formation with cyclobutyl piperidine provided final compound **6t**.

The construction of spirocyclobutyl analogues is detailed in Scheme 7. The synthesis began with *tert*-butyl 4-methylenepiperidine-1-carboxylate **18**, which was converted to dichloroketone **19** by reaction with Cl_3COCl. The chlorine atoms were subsequently removed with Zn to provide ketone **20**. The ketone was then reduced with $NaBH_4$ and converted to the mesylate, providing intermediate mesylate **21**. The mesylate was then displaced with cyanide and hydrolyzed to give Boc-protected ester **23**.

Preparation of final analogues on the cyclobutyl scaffold is shown in bottom synthetic pathway of Scheme 7. Boc-protected ester **23** was deprotected and subjected to reductive amination with dihydro-2*H*-pyran-4-(3*H*)-one under catalytic hydrogenation to give intermediate ester **28**. Hydrolysis and amide bond coupling with varying piperazines provided final compounds **29a–f**.

An alternative synthesis illustrated in Scheme 8 was employed to provide intermediates for analogues on the cyclobutyl scaffold. Cbz-protected olefin **2** (previously described in Scheme 1) was reacted with dichloroketene precursor to give intermediate dichloroketone **24**. This was reduced to ketone **25** with Zn and then converted to aldehyde **26** by Wittig olefination. The final intermediate was then obtained by 2,2,6,6-tetramethylpiperidine oxide (TEMPO) oxidation to provide acid **27**.

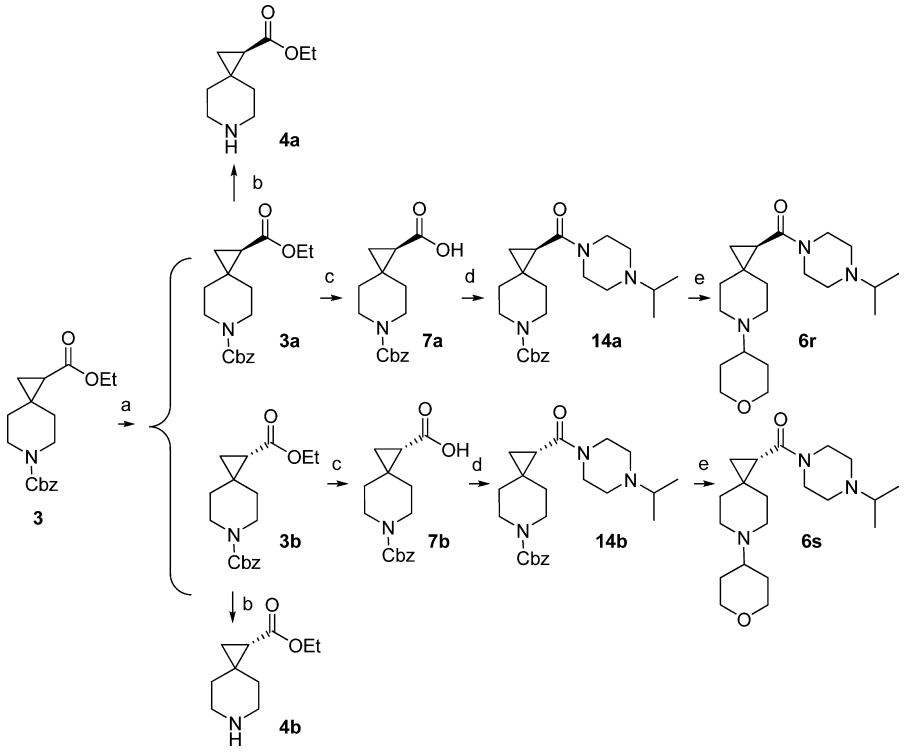
Intermediate **27** was utilized to explore substitution projecting from the piperidine nitrogen atom. The route employed for these compounds is shown in the bottom synthetic pathway of Scheme 8. Intermediate **27**[27] was reacted with 1,4-cyclobutyl piperazine and 1,4-cyclobutyl diazepane to form intermediate Cbz-protected piperazines **30g** and **30j**. The Cbz group was removed, and various coupling strategies were employed to derivatize the N atom, such as sulfonamide formation (**29g**), Buchwald–Hartwig amination (**29h**),[25] and acylation (**29i** and **29j**).

An azetidine analogue was prepared according to Scheme 9. Spirofused azetidine **32** was acylated with *p*-nitrophenyl carbonochloridate and then reacted with isopropyl piperidine to provide intermediate **33**. This intermediate was converted to final compound **34** under the reductive amination conditions previously described in Scheme 8.

Spirocyclic fused pyrrolidines attached to the 3 and 4 positions of the piperidine ring are illustrated in Schemes 10

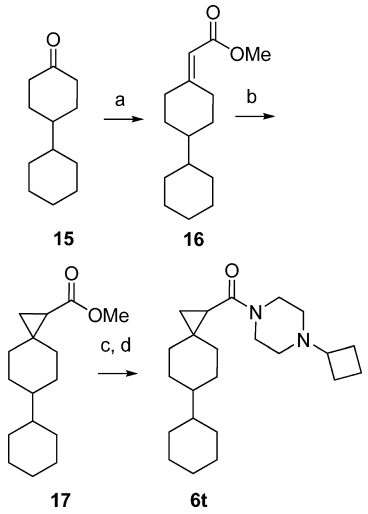
dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX–XXX

Scheme 5[a]



[a]Reagents and conditions: (a) Chiral SFC, quan.; (b) Pd/C, H_2, EtOH, rt, 59% for **4a** and 82% for **4b**; (c) NaOH, MeOH, H_2O, quan; (d) HBTU, 1-isopropylpiperazine, quan.; (e) Pd/C, H_2, MeOH, dihydro-2*H*-pyran-4-(3*H*)-one, 30 psi, 45% for **6r** and 68% for **6s**.

Scheme 6[a]



[a]Reagents and conditions: (a) THF, NaH, $(MeO)_2PCH_2CO_2Me$, cyclohexanone, 72%; (b) DMF, NaH, $Me_3S^+I^-$, 49%; (c) LiOH, THF, quan.; (d) DMF, HBTU, DIPEA, cyclobutylpiperazine, 69%.

and 11. For N-tetrahydropyran, isopropyl piperidine, and diazepane analogues, the synthesis began with spirofused intermediate **35** or **39** (Scheme 10). The free nitrogen of **35** was acylated with carbonyldiimidazole (CDI), and the intermediate was methylated with methyl iodide and then reacted with 1-isopropylpiperazine to give **36b**. This was converted into the final test compounds through reductive amination to provide **37b**. Access to the 4-piperidine spirofused cyclopentanes began with **39**. This was converted to the intermediate urea by reaction with *p*-nitrophenyl carbonochloridate and 1,4-isopropyl piperazine. Deprotection of the Boc group with HCl followed by reductive amination provided **41a**. In an analogous fashion, Scheme 11 illustrates the synthesis of cyclobutyl piperidines and diazpeines for the 3- and 4-substituted piperidines. In this set, several N substituents were explored, such as N-acyl and N-sulfonyl, following the chemistry outlined in Scheme 11.

Scheme 12 illustrates the synthetic protocol used to access the piperidine replacement of the right-hand-side piperazine. Boc-protected spirofused intermediate **42** was acylated with benzoyl chloride to provide **43**, which then underwent coupling with 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid to yield intermediate **44**. This intermediate was subjected to reductive amination with cyclobutanone to give final compound **41g**.

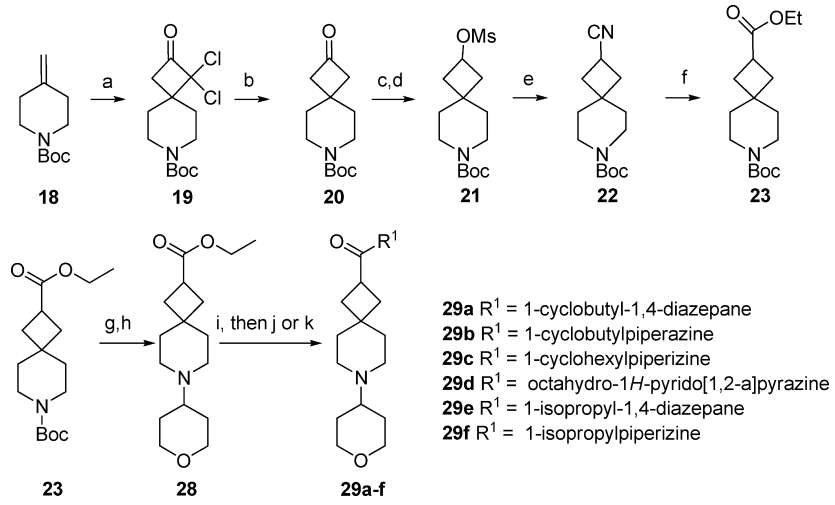
The final synthetic protocol is shown in Scheme 13, which illustrates the route employed to gain access to the spirofused bis-piperidine scaffold. The scheme was analogous to that used in Schemes 10 and 11, whereby starting spirofused amine **45** was converted to urea **46** to provide final compound **41h** using protocols outlined in Scheme 13.

RESULTS AND DISCUSSION

Initial investigations in this program began with the 6-azaspiro[2.5]octane (spirocyclopropyl) core reported in Table 1. Functional data was determined using an agonist-stimulated [^{35}S]GTPγS (GTPγS) binding assay with membranes from CHO-K1 cells stably transfected with the long form of the human H_3R (445 amino acids).[28] To complement the functional assay, a binding assay was also utilized that measured the ability of test compounds to displace [^{3}H]-*N*-α-methylhist-

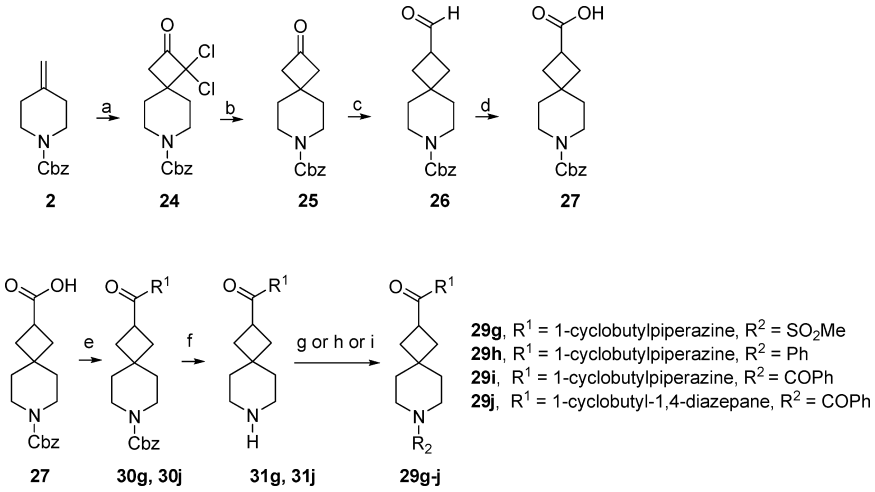
dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 7[a]



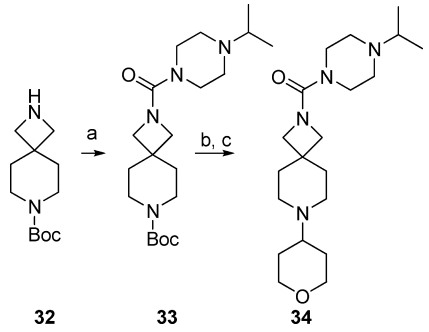
[a]Reagents and conditions: (a) Zn−Cu, Et_2O, Cl_3COCl, 40%; (b) NH_4Cl, MeOH, Zn, 97%; (c) $NaBH_4$, MeOH, 99%; (d) Et_3N, MsCl, CH_2Cl_2, 97%; (e) NaCN, TBAB, DMF, 57%; (f) EtOH, H_2SO_4; Boc_2O, 41%; (g) TFA, CH_2Cl_2, rt, 83%; (h) MeOH, dihydro-2*H*-pyran-4-(3*H*)-one, Pd/C, H_2, rt, 58%; (i) MeOH, 1 N NaOH, reflux, quan.; (j) DMF, HBTU, amine, DIPEA, 76%; (k) CO_2Cl_2, CH_2Cl_2; DIPEA, R^1-amine, CH_2Cl_2, 32−65%.

Scheme 8[a]



[a]Reagents and conditions: (a) Et_2O, Zn−Cu, Cl_3COCl, DME, 40%; (b) MeOH, NH_4Cl, Zn, 74%; (c) THF, KO*t*-Bu, BuOH, $Ph_3PCH_2OMe^+Cl^-$, 48%; (d) CH_2Cl_2/$NaHCO_3$, NaOCl, TEMPO, NaBr, 72%; (e) DMF, DIEA, amine, HBTU, 78% or SO_2Cl_2, DIEA, CH_2Cl_2, quan.; (f) EtOH, Pd/C, H_2 50 psi, 88−96%; (g) CH_2Cl_2, TEA, $MeSO_2Cl$, 39%; (h) PhMe, Ph-Br, $Pd(OAc)_2$, Cs_2CO_3, BINAP, 110 °C, 18h, 46%; (i) CH_2Cl_2, DIPEA, PhCOCl, 33−64%.

Scheme 9[a]



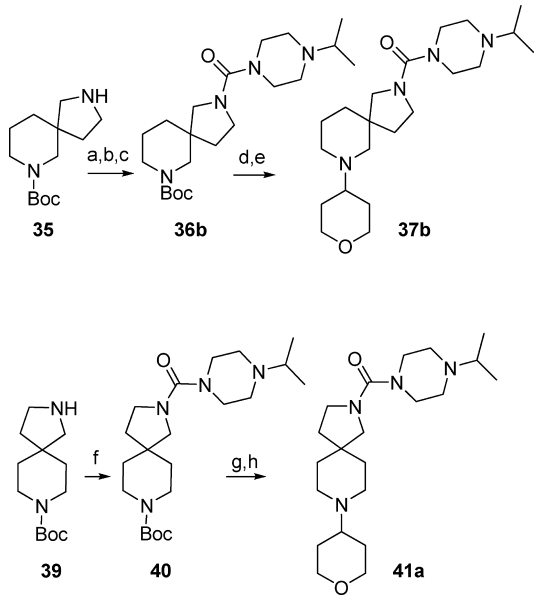
[a]Reagents and conditions: (a) THF, 4-NO_2PhOCOCl, DIPEA, 1-isopropylpiperazine, reflux, 72%; (b) HCl, MeOH, rt, quan.; (c) MeOH, TEA, dihydro-2*H*-pyran-4-(3*H*)-one, Pd/C, H_2, rt, 28%.

amine binding to human H_3R-K1 membranes.[29] Because of the good correlation of the two assays and the translational importance of the functional assay, the functional GTPγS assay became the only SAR-driving assay utilized for the project, with an occasional confirmatory test using the binding assay. This series of H_3 antagonists was not routinely assessed for inverse-agonist activity. However, **6d** reduced basal GTPγS binding to membranes expressing recombinant human H_3R, which is consistent with inverse-agonist activity and has been reported for other H_3 antagonists.[14] Because the pharmacophore possesses a lipophilic basic amine and prior H_3 compounds were known to have a history of hERG activity, the hERG data (electrophysiological measurements in CHO cells) is also reported even though only a few compounds in the series ever demonstrated even a modest signal.

Table 1 highlights the importance of the SAR of the N-alkyl piperazine substituent. N-Me derivative **6a** has relatively weak

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

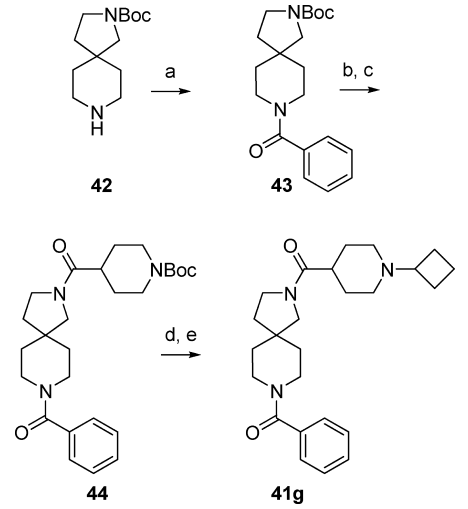
Scheme 10[a]



[a]Reagents and conditions: (a) THF, CDI, Et_3N; (b) ACN, MeI; (c) CH_2Cl_2, R^1-amine, Et_3N, 40% steps a–c; (d) HCl, *i*PrOH, Et_2O, quan.; (e) $NaBH(OAc)_3$, 1,2-DCE, dihydro-2*H*-pyran-4-(3*H*)-one, AcOH, Et_3N, 40–44%; (f) THF, 4-NO_2PhOCOCl, −78 °C, 1-isopropylpiperazine, reflux, DIPEA, 28%; (g) HCl, MeOH, 75%; (h) MeOH, TEA, dihydro-2*H*-pyran-4-(3*H*)-one, Pd/C, H_2, rt, 62%.

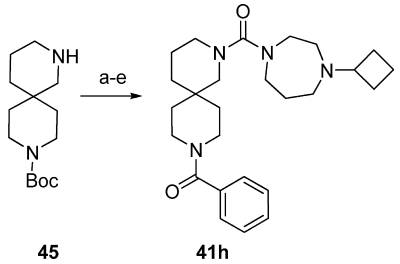
activity in both the functional and binding assays (1100 and 1300 nM, respectively). The addition of isopropyl (**6b**), cyclopropyl (**6c**), and cyclobutyl (**6d**) led to functional activity in the double- and single-digit nanomolar region, with no discernible hERG activity (GTPγS IC_{50} = 3.2, 19, and 3.6 nM, respectively). The isopropyl and cyclobutyl appeared optimal, as moving to larger groups such as cyclohexyl (**6f**) and cycloheptyl (**6g**) demonstrated a subtle but gradual loss of functional activity (GTPγS IC_{50} = 12 and 74 nM, respectively). Polar modifications at the terminal N-alkyl piperazine, such as N-4-pyridyl (**6i**), also led to decreased activity relative to the small alkyl derivatives. Compound **6j** was an attempt to understand the promiscuity of the pharmacophore by swapping

Scheme 12[a]



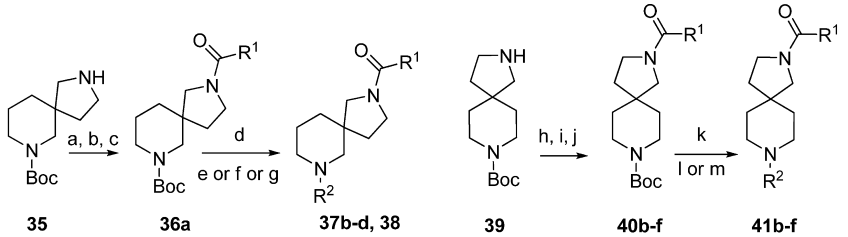
[a]Reagents and conditions: (a) 2-MeTHF, $PhCO_2H$, DIPEA, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-4-ium, 61%; (b) HCl, MeOH, quan.; (c) 2-MeTHF, DIPEA, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-4-ium, 1-(*tert*-butoxycarbonyl)-piperidine-4-carboxylic acid, 22%; (d) dioxane, HCl, 64%; (e) DMF, $NaCNBH_4$, cyclobutanone, 37%.

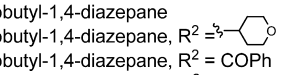
Scheme 13[a]



[a]Reagents and conditions: (a) THF, CDI, Et_3N, quan.; (b) ACN, MeI, quan.; (c) CH_2Cl_2, Et_3N, 1-cyclobutyl-1,4-diazepane, 80%; (d) HCl, *i*PrOH, quan.; (e) benzoyl chloride, CH_2Cl_2, Et_3N, 75%.

Scheme 11[a]



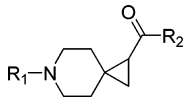
36a, R^1 = 1-cyclobutyl-1,4-diazepane
37a, R^1 = 1-cyclobutyl-1,4-diazepane, R^2 = 
37c, R^1 = 1-cyclobutyl-1,4-diazepane, R^2 = COPh
37d, R^1 = 1-cyclobutyl-1,4-diazepane, R^2 = SO_2Ph
38, R^1 = 1-cyclobutyl-1,4-diazepane, R^2 = H

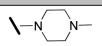
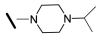
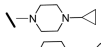
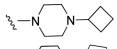
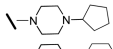
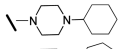
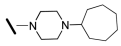
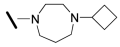
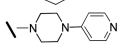
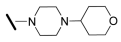
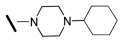
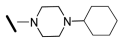
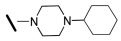
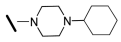
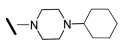
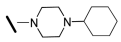
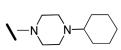
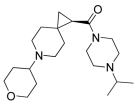
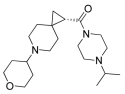
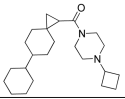
41b, R^1 = 1-cyclobutyl-1,4-diazepane, R^2 = COPh
41c, R^1 = 1-cyclobutyl-1,4-diazepane, R^2 = SO_2Ph
41d, R^1 = 1-cyclobutyl-1,4-diazepane, R^2 = SO_2*i*-Pr
41e, R^1 = 1-cyclobutylpiperazine, R^2 = COPh
41f, R^1 = 1-cyclobutylpiperazine, R^2 = SO_2Ph

[a]Reagents and conditions: (a) THF, CDI, Et_3N; (b) ACN, MeI; (c) CH_2Cl_2, R^1-amine, Et_3N, 40% steps a–c; (d) HCl, *i*PrOH, Et_2O, quan.; (e) $NaBH(OAc)_3$, 1,2-DCE, dihydro-2*H*-pyran-4-(3*H*)-one, AcOH, Et_3N, 36%; (f) CH_2Cl_2, PhCOCl, TEA, 48%; (g) $PhSO_2Cl$, TEA, 50%; (h) THF, CDI, Et_3N, quan.; (i) ACN, MeI, quan.; (j) CH_2Cl_2, R^1-amine, Et_3N, 65–67%; (k) HCl, *i*PrOH, quan.; (l) CH_2Cl_2, PhCOCl, TEA, 85–88%; (m) R_2SO_2Cl, TEA, 77–85%.

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX–XXX

Table 1. Spirocyclopropyl Analogues



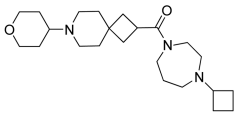
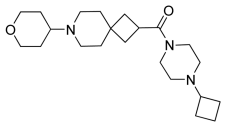
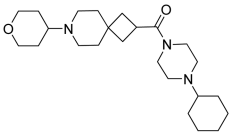
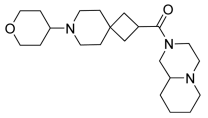
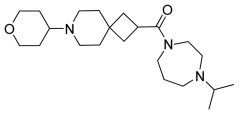
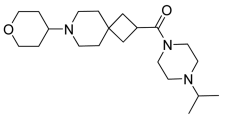
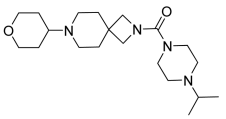
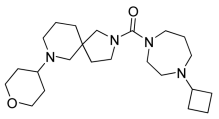
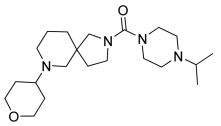
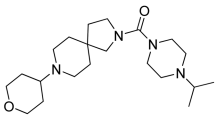
Entry	R1	R2	hH_3 GTPγS (IC_{50}, nM)[a]	hH_3 SPA (K_i, nM)[b]	hERG (IC_{50} μM)[c]	logD[d]
6a			1100	1300	nm	nm
6b			3.2	3.0	>33	nm
6c			19	11	>33	nm
6d			3.6	1.3	>33	0.24
6e			8.8	1.4	>33	nm
6f			12	26	>33	0.74
6g			74	65	nm	nm
6h			17	3.0	nm	0.18
6i			140	8.4	nm	-1.09
6j			66	67	nm	nm
6k	H		360	439	>33	-1.00
6l			10	19	20.8	nm
6m			19	47	nm	0.74
6n			nm	1670	nm	3.63
6o			nm	103	nm	2.81
6p			16	21	nm	nm
6q			83	55	nm	1.09
6r			87	16	>33	-0.35
6s			8.3	0.8	>33	-0.26
6t			nm	>10 mM	nm	nm

[a]Compounds were tested for activity at inhibiting agonist-stimulated [^{35}S]GTPγS binding to membranes from CHO-K1 cells stably transfected with the long form of the H_3 receptor (445 amino acids). Results are an average of $n > 2$ with standard error <20%. [b]SPA binding assay utilized hH_3 transfected CHO membranes and [^{3}H]-*N*-α-methylhistamine. Results are an average of $n > 2$ with standard error <50%. [c]Values determined in CHO-K1-hERG cells using electrophysiological measurements. [d]logD values between octanol and water, as determined by shake-flask HPLC methods. nm, not measured.

the N-alkyl side pieces relative to **6d**. A loss of ∼18-fold functional activity relative to **6d** was observed, suggesting that a proper orientation of these two pieces significantly contributes to the binding and functional activity. A series of analogues was explored where the N-alkyl piperazine derivative was fixed as a cyclohexyl group (**6k**−**q**), and the piperidine N-alkyl group was subsequently explored. The absence of an N-alkyl group (**6k**) led to a 30-fold drop in activity relative to **6f**. Replacement of the tetrahydro-2*H*-pyran in **6f** with a cyclohexyl (**6l**) gave an equipotent compound; however, hERG activity began to appear in the series for the first time (20.8 μM). This observation led us to conclude that having two hydrophobic groups, one off of the piperidine N atom and one off the piperazine N atom, led to deterioration of the clean off-target profile in compounds such as **6f** and hence having a polar group off the piperidine N atom was important for avoiding these issues. Smaller hydrophobic groups such as isopropyl **6m** were also tolerated. Addition of an aromatic ring at varying distances was also explored. Direct attachment of a phenyl ring (**6n**) led to a compound with poor binding activity (1670 nM), and as a result, the functional activity was not measured. This compound is expected to be a monobasic compound, given the pK_a of the aniline nitrogen makes it unlikely to be protonated at physiological pH. However, examples shown later in Table 2 illustrate that certain monobasic compounds can have good potency. The reason for the lack of activity for the phenyl (**6n**) is unclear, but we speculate it has more to do with a steric interaction than with a lack of protonatable amine at the left-hand-side piperidine. Benzyl-linked compound **6o** demonstrated more potent H_3 binding than the phenyl analogue (103 nM), although it was still not as potent as polar tetrahydro-2*H*-pyran analogue **6f**. Extension to the phenethyl analogue **6p**

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

Table 2. Azetidine and Pyrrolidne Analogues with N-Tetrahydropyran-2*H*-pyran

Entry	R1	R2	hH_3 GTP-γ-S (IC_{50}, nM)[a]	hERG IC_{50} (μM)[b]	logD[c]
29a			0.8	nm	-0.89
29b			1.9	>33	nm
29c			1.6	nm	0.75
29d			6.3	>33	-0.46
29e			14	nm	-1.78
29f			1.7	>33	-0.74
34			900	nm	nm
37a			4.4	>33	0.23
37b			140	>33	-0.19
41a			32	>33	-0.87

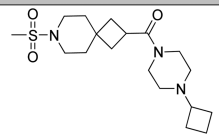
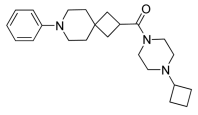
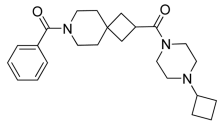
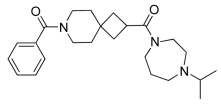
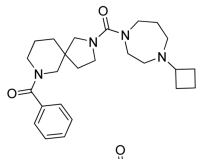
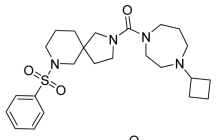
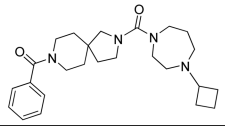
[a]Compounds were tested for activity at inhibiting agonist-stimulated [^{35}S]GTPγS binding to membranes from CHO-K1 cells stably transfected with the long form of the H_3 receptor (445 amino acids). Results are an average of $n > 2$ with standard error <20%. [b]Values determined in CHO-K1-hERG cells using electrophysiological measurements. [c]logD values between octanol and water, as determined by shake-flask HPLC methods. nm, not measured.

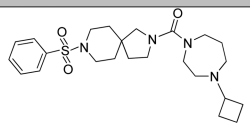
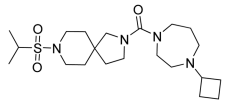
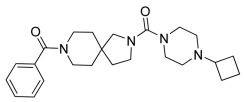
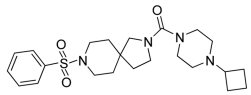
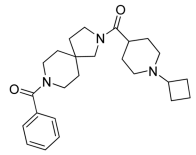
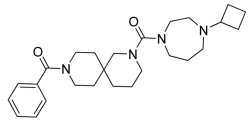
gave activity more comparable to **6f**. Replacement of the tetrahydro-2*H*-pyran with a 4-pyridyl (**6q**) led to a compound with only a slight change from initial starting point **6f** as well, further reinforcing the promiscuity of this pocket with respect to binding and functional activity. This promiscuity thus provided opportunities to identify the best substituent from a pharmacokinetic and off-target perspective while maintaining intrinsic activity at the H_3 receptor.

The separation and assignment of enantiomers of **6b** was conducted by separation on chiral SFC chromatography of intermediates **3a** and **3b** and assignment based on calculated and measured VCD spectra.[26] (*S*) stereoisomer **6s** demonstrated a more favorable functional and binding profile compared with (*R*) stereoisomer **6r** (GTPγS IC_{50} = 8.3 and 87 nM and binding IC_{50} = 0.8 and 16 nM, respectively). Compound **6s** was thus selected as a tool compound to

dx.doi.org/10.1021/jm4014828 | *J. Med. Chem.* XXXX, XXX, XXX−XXX

Table 3. Spirofused Pyrrolidine and Piperidines with N Substituents

Cpd	R1	hH3 GTPγS IC_{50} (nM)[a]	hERG IC_{50} (μM)[b]	logD[c]
29g		11	>33	0.97
29h		66	>33	3.25
29i		48	>33	2.09
29j		12	>33	1.63
37c		4.0	nm	1.01
37d		6.5	>33	1.74
41b		3.6	>33	1.22

Cpd	R1	hH3 GTPγS IC_{50} (nM)[a]	hERG IC_{50} (μM)[b]	logD[c]
41c		2.7	>33	1.71
41d		3.2	>33	0.80
41e		13	>33	1.80
41f		21	nm	2.27
41g		340	nm	
41h		18	nm	1.62

[a]Compounds were tested for activity at inhibiting agonist-stimulated [^{35}S]GTPγS binding to membranes from CHO-K1 cells stably transfected with the long form of the H_3 receptor (445 amino acids). Results are an average of $n > 2$ with standard error <20%. [b]Values determined in CHO-K1-hERG cells using electrophysiological measurements. [c]logD values between octanol and water, as determined by shake-flask HPLC methods. nm, not measured.

benchmark the pharmacokinetic and in vivo efficacy of this scaffold. Finally, a piperidine replacement, 4-cyclohexyl **6t**, was synthesized and tested and demonstrated a complete loss of activity (>10 uM), thus reinforcing the importance of a heteroatom in the left-hand-side piperidine position. Furthermore, the impact of solubility on this change was dramatic, as might be expected, with **6t** having a measured solubility of ∼1 μM (data not shown), whereas most other piperidine analogues were >500 μM.

The SAR was next investigated on a 7-azaspiro[3.5]nonan-2-yl core (spirocyclobutyl) and is reported in Tables 2 and 3. In the spirocyclobutyl series, piperazine modifications on right-hand-side **29a**, **29b**, and **29c** provided very potent functional antagonists (GTPγS IC_{50} = 0.8, 1.9, and 1.6 nM, respectively). In comparison to the spirocyclopropyl matched pairs **6h**, **6d**, and **6f**, all three spirocyclobutyl compounds were more potent (cf. GTPγS IC_{50} = 17, 3.6, 12 nM, respectively, for spirocyclopropyl vs spirocyclobutyl matched pairs). One possible explanation could be that the spirocyclopropyl analogues are all racemic (whereas the achiral spirocyclobutyl is not), so potentially pure separated spirocyclopropyl stereoisomers could be more potent (but only theoretically up to a factor of 2). Because the spirocyclobutyl series is achiral, it proved advantageous over spirocyclopropyl compounds with regards to time spent on chiral separation and characterization. Bicyclic variant **29d** demonstrated comparable potency to the acyclic analogues, suggesting that there was room for further expansion at the α-position of the piperazine ring. Matched pairs **29e** and **29f** were designed to understand the impact of piperazine compared to 1,4-diazepane, where the isopropyl group was held constant. The results indicated a negative impact on the larger core (1,4-diazepane) and was consistent with the trends observed in the spirocyclopropyl series (**6d** vs **6h**). These results are inconsistent with the equipotent matched pairs of **29a** and **29b** mentioned earlier, which suggests that there is a unique trajectory and binding mode utilized by the spirocyclobutyl core with an N-cyclobutyl right-hand-side chain. Azetidine analogue **34** is an N-matched-pair variant of **29f** that demonstrated a significant loss in potency compared with **29f** (GTPγS IC_{50} = 900 vs 1.7 nM, respectively). This trend was not observed with pyrrolidine analogues **37a**, **37b**, and **37c** (Table 3). N-isopropyl derivative

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

37b is ∼5−6-fold more potent than comparator azetidine analogue **34**. A more dramatic increase in potency is observed on switching to 1,4-diazepane N-cyclobutyl **37a** (GTPγS IC_{50} = 4.4 nM). Exploration of the spirocyclic 4-pyrrolidine core modification led to **41a** (GTPγS IC_{50} = 32 nM), which was ∼4-fold more potent than spirocyclic 3-pyrrolidine matched pair **37b**.

For **29g−I** (Table 3), the N-cyclobutyl piperazine was held constant, and series of N-sulfonyl (**29g**), N-phenyl (**29h**), and N-benzoyl (**29i**) derivatives were made. All of these derivatives were less potent than N-tetrahydro-2*H*-pyran comparator **29b** by ∼10−30-fold, as measured in the GTPγS functional assay. The N-isopropyl 1,4-diazepane with the N-benzoyl attachment to the piperidine ring was prepared (**29j**) and demonstrated equal potency to N-tetrahydro-2*H*-pyran comparator **29e** (GTPγS IC_{50} = 12 vs 14 nM). N-linked spirocyclic modifications such as azetidine, 3-pyrrolidine, 4-pyrrolidine, and 4-piperidine were next explored. Replacement of the N-tetrahydro-2*H*-pyran in **37a** with N-benzoyl **37c** gave a compound with equal potency (GTPγS IC_{50} = 4.0 nM). Replacement of the N-benzoyl with N-phenylsulfonyl (**37d**) also led to a compound with similar potency (GTPγS IC_{50} = 6.5 nM), suggesting a wide tolerance of functional groups with the 3-pyrrolidine-1,4-diazepane-N-cyclobutyl motif. Extension of the SAR with N-benzoyl, N-phenyl sulfonyl, and N-isopropylsulfonyl (**41b**, **41c**, and **41d**) led to compounds with single-digit nanomolar potency comparable to spirocyclic 3-pyrroldine matched pairs **37c** and **37d**. (N-isopropylsulfonyl was not made in the 3-pyrrolidine series.) Analogues **41e** and **41f** were made to explore the comparison of N-1,4-cyclobutyl piperazine to N-1,4-cyclobutyl diazepane with the N-benzoyl and N-phenylsulfonyl groups attached on the piperidine. The N-1,4-cyclobutyl piperazine was not as potent as the analogues with the diazepane ring (**41e**, GTPγS IC_{50} = 13 nM and **41f**, GTPγS IC_{50} = 21 nM compared with 3.6 and 2.6 nM for matched pairs with the diazepane ring). Compound **41g** was prepared to examine replacement of the piperazine ring with a piperidine ring. A significant drop in activity was observed (GTPγS IC_{50} = 340 nM). The 4-piperidine right-hand side is expected to have less of a planar geometry than the corresponding piperazine ring, perhaps suggesting the importance of this as a spatial requirement. Finally, 4-spirocyclic piperidine ring core modification **41h** was prepared and demonstrated ∼6-fold drop in activity compared to 4-spirocyclic pyrrolidine analogue **41b**.

Various molecular overlays were derived from conformations generated with molecular mechanics methods (see Computational Spectral Simulations) and were used to help to try to understand the topological features that may be essential for activity. Conessine was chosen as a useful benchmark (hH_3 K_i binding = 5.4 nM, hH_3 (GTPγS) IC_{50} = 11 nM)[18b] given its structural rigidity and availability of an X-ray structure.[18c] Compound **6s** (hH_3 K_i binding = 0.8 nM, hH_3 IC_{50} (GTPγS) = 8.3 nM) was found to have low-lying conformations with appropriately placed diamines that mapped well to the structure of Conessine (Figure 2a). Three other compounds were modeled (**29f**, **34**, and Bavisant; Figure 2b), and energetically reasonable conformers were found in each case to superimpose on one another. This set of structures highlights that even though a gross approximation of pharmacophore match can be gained from these types of analyses, the granularity of activity may not be easily discerned. For example, **34** is weakly active (hH_3K_i binding = 97 nM, hH_3 (GTPγS) IC_{50} = 900 nM) compared to **29f** (K_i binding = 0.3 nM, hH_3 (GTPγS) IC_{50} = 1.7 nM) and Bavisant (example of a potent, conformationally constrained clinical candidate with reported in vitro K_i binding = 5.4 nM).[13f] The three structural overlays of these molecules can be found that show very little distinction. It is possible that either unanticipated electronic changes happen between the cyclobutyl and azetinde linkers or that further subtleties of the substantially large set of low-lying conformers exist, and simple overlays based only on access to low-lying conformations are not an accurate predictor of the bioactive conformation or potential excluded volume for this linker. Furthermore, the model would be most useful in predicting binding energetics and not functional activity because the protein conformational changes that must occur for a binding ligand to become a functional ligand are unknown. From a binding perspective, it may not be possible to distinguish potent binders (e.g., **29f** K_i = 0.3 nM and **34** K_i = 97 nM) with a simple conformational overlay approach. Rank ordering with this approach was not possible, but it did serve to help understand the general pharmacophore features within a loose-fit model.

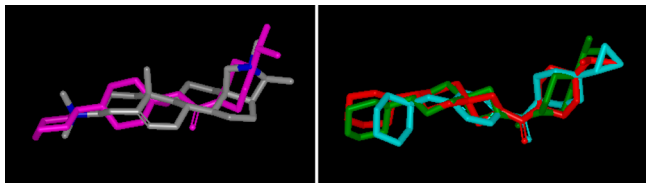


Figure 2. (left) Molecular overlays of Conesine (gray, small-molecule X-ray structure) and **6s** (purple, low-lying conformation). (right) Molecular overlays of **29f** (red), **34** (green), and Bavisant (light blue).

A subset of compounds was examined by in vitro DMPK profiling, which is summarized in Table 4. All of the compounds tested had high solubility (>500 μM) and significant free-drug concentrations, as determined in both rat and human plasma protein binding assays (∼50−90% free in most cases). Most compounds demonstrated high permeability, as measured in the Madin-Darby canine kidney (MDCK) permeability assay, except for an occasional molecule, such as **6e**, which showed modest efflux and low permeability relative to other compounds. In general, most compounds were not significantly metabolized by rat or human microsomes, with many compounds measuring <4 μL/min/mg. Again, compound **6e** was an outlier, demonstrating relatively higher rat clearance (43 μL/min/mg). It is not clear why the pyrrolidine ring on the right-hand-side piperazine results in these modest changes on these properties. None of the compounds tested demonstrated any significant inhibition of cytochrome P450 enzymes in a representative panel (CYP2D6, CYP3A4, and CYP2C9). There was no observed difference between separated chiral isomers and racemic compounds in this panel of in vitro DMPK assays; thus, racemic compounds were tested for expediency. Measured logD values are reported in Tables 1−3. In general, the logD varied as expected. For example, the tetrahydropyran substituent contributed to low and sometimes negative logD values (e.g., **6s**, logD = −0.26 and **6i**, logD = −1.09), and hydrophobic substituents gave higher logD values (e.g., **29h** N-phenyl, logD = 3.25 and **29i** N-benzoyl, logD = 2.09). However, it did not appear that the increasing logD contributed substantially to any increased hERG liability or in vitro DMPK liability because most of the compounds, regardless of logD value, maintained low hERG and in vitro DMPK liabilities.

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

Table 4. In Vitro DMPK

entry	sol[a]	hPPB[b]	rPPB[c]	MDCK perm[d]	efflux[e]	hClint[f]	rClint[g]	CYP 2D6 (IC_{50})[h]	CYP 3A4 (IC_{50})[h]	CYP 2C9 (IC_{50})[h]
6b	>500	87	69	5.4	1.8	<4	24	>20	nm	>20
6c	>500	nm	nm	8.4	1.5	<4	20	>20	nm	nm
6d	>500	91	80	3.9	3.3	29	<4	>20	nm	>20
6e	>500	50	65	<2.2	>5.5	7.2	43	>20	nm	>20
6l	>500	81	62	<1.7	>2.0	14	31	>20	>20	nm
6s	>500	nm	87	13	0.7	<4	7	>20	>20	>20
29b	>500	51	nm	5.1	6.2	<4	<4	>20	>20	nm
29f	>500	nm	nm	3.6	3.3	<4	<4	>20	>20	>20
29g	>500	67	nm	19	1.5	8.3	16	>20	>20	>20
41d	>500	nm	nm	7.8	6.0	20	34	>20	>20	>20
41e	>500	nm	nm	8.8	4.5	18	25	>20	>20	>20

[a]Equilibrium solubility (pH 7.4). [b]Human plasma protein binding expressed as the percent free. [c]Rat plasma protein binding expressed as the percent free. [d]Permeability measured in MDCK cell lines (A to B P_{app} 1×10^{-6} cm/s). [e]Efflux as measured in MDCK cell lines (ratio of A-to-B P_{app} over B-to-A P_{app}). [f]Human liver microsomal clearance expressed as μL/min/mg. [g]Rat liver microsomal clearance expressed as μL/min/mg. [h]In vitro inhibition of cytochrome P450 CYP2D6, CYP3A4, and CYP2C9 isoforms (IC_{50} values).

Compounds were profiled in a small panel of off-target receptors as well as against closely related histamine receptors. A sampling of some of these receptors is shown in Table 5.

Table 5. Representative Off-Target Activity

target[a]	**29b** (activity at 10 μM)	**6s** (activity at 10 μM)
H_1	>10	>10
H_2	nt	>10
H_4	>10	>10
A2a	>10	>10
5HT1A	>10	>10
NAchE	>10	>10
NET	>10	>10
M_2	>10	>10
D_2	>10	>10
GABA	>10	>10
PDE_4	>10	>10

[a]Representative binding values at 10 μM concentration against a sampling of targets (Ricerca testing laboratories).

Neither **29b** nor **6s** demonstrated activity against the other three histamine receptors (H_1, H_2, and H_4) or other biogenic amine receptors sometimes seen with basic and hydrophobic motifs. Compound **6s** was further tested in expanded panel of 144 in vitro assays to assess any off-target liabilities (see Supporting Information for panel list). The only hit from these screens was modest σ2 receptor activity for **6s** (62% at 10 μM), with the remainder below the defined criteria for activity for that particular assay at 10 μM single-concentration testing. Compound **6s** was also found to be inactive in an Ames mutagenicity screen as well as in a phospholipidosis assay (EC_{50} >300 μM).[30] Phospholipidosis (PLD) is a concern among the lipophilic basic amine scaffolds prominent in the H_3 field[31] and has been extensively studied by the J&J group. As an example, compounds such as JNJ-5207852 can be predicted on the basis of their in vitro PLD values to predict in vivo PLD findings.[32] The logD was measured for **6s** and found to be −0.26, which was consistent with many compounds in the dibasic series. Representative logD values are recorded in Tables 1−3. It was speculated that the low logD in the dibasic motif gave rise to the lack of activity in the PLD assay; however, compounds of higher logD were not tested in the PLD assay, which would have provided a stronger basis for this hypothesis. On the basis of the positive in vitro DMPK and off-target profile, compounds **29b** and **6s** were followed up with in vivo studies.

Pharmacokinetics. Table 6 summarizes the in vivo pharmacokinetic (PK) data on six representative compounds. All of the dibasic compounds (including **6s** mentioned earlier) had very high volumes of distribution and long half-lives. For example, **29b** and **29f** had calculated volumes of distribution of 74 and 64 L/kg, respectively, with half-lives >10 h. For those compounds with this profile, the long half-life did not allow for precise characterization of the volume of distribution. Compound **6s** demonstrated the lowest rate of clearance of the three dibasic compounds by a factor of ∼4. The monobasic compounds, such as **29g**, **41d**, and **41e**, all demonstrated much lower half-lives and volumes of distribution (e.g., **29g** V_{ss} 1.44 L/kg and $t_{1/2}$ = 0.40) but had higher rates of clearance than **6s**. The high volume of distribution and long half-life for dibasic compounds has been reported in the H_3 area; for example, JNJ 5207852 is reported to have similar characteristics.[19]

A more comprehensive evaluation of pharmacokinetics and central nervous system (CNS) penetration of test compound

Table 6. In Vivo DMPK and PK Parameters

Cpd	V_{ss} (L/kg)[a]	$t_{1/2}$ (h)[b]	Clint (mL/min/kg)[c]	n[d]	dose (μmol/kg)[e]	charge[f]
6s	34.6 ± 1.5	25.3 ± 0.6	16.4 ± 0.8	4	3.0	dibasic
29b	74.0 ± 9.1	14.2 ± 3.9	81.2 ± 17.6	3	2.4	dibasic
29f	64	11.7	78	1	2.1	dibasic
29g	1.4 ± 0.1	0.4 ± 0.1	69.8 ± 5.6	7	3.5	monobasic
41d	2.1 ± 0.1	0.6 ± 0.1	53 ± 0.9	2	1.8	monobasic
41e	3.7 ± 0.2	1.1 ± 0.1	91 ± 3.7	4	2.3	monobasic

[a]Mean volume of distribution measured after IV dosing in male fasted rats. [b]Mean half-life as measured from IV dosing in rats. [c]Mean clearance as measured from IV dosing in rats. [d]Number of rats used in the experiment. [e]Dose administered. [f]Number of predicted basic groups.

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

6s was completed in male Sprague–Dawley rats (Figure 3). The plasma clearance calculated on the basis of area under the curve (AUC) from 0–24 h was high at 53 mL/min/kg. This represents an overestimate of the true clearance, which is likely to be in the moderate range but would require longer sampling times to characterize fully. The volume of distribution was very high and the apparent half-life was long at 15 h, but these parameters could not be precisely characterized with confidence using a 24 h sampling protocol. The oral bioavailability, estimated on the basis of comparison of the AUC (0–10 h) following IV and oral administration, was high at 99%. Approximately 14% of the dose was recovered in the urine during the first 24 h following intravenous bolus administration. Compound **6s** distributed into the CNS as demonstrated by a brain/plasma ratio of 3.0 and cerebrospinal fluid (CSF)/plasma ratio of 0.19 (Table 7).

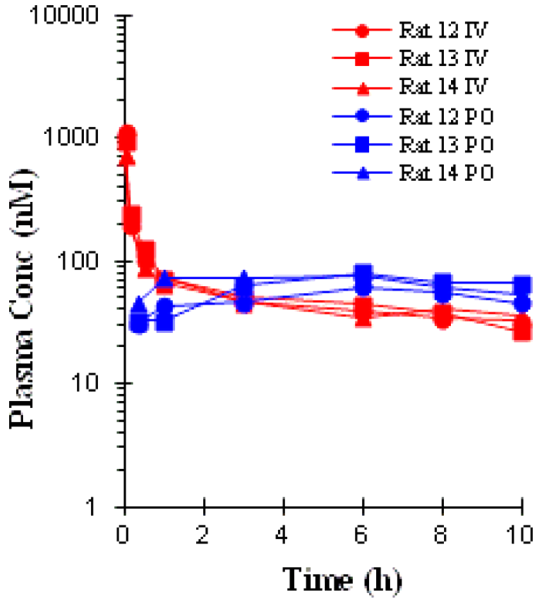


Figure 3. Plasma concentrations and arterial pharmacokinetic parameters of **6s** following a 3 μmol/kg IV (fasted rats) dose and 3 μmol/kg PO (fed rats) dose in male Sprague–Dawley rats using a crossover study design with a PO formulation of 10% DMA and 18% SBECD, pH 4.73.

In Vivo Efficacy. Compound **6s** was tested for activity in a mouse model of learning and recognition memory.[33] Briefly, male CF-1 mice were placed in a test environment that contained two identical objects and were allowed to explore the environment (acquisition phase). The mice were removed from the environment, and one of the objects was replaced with a novel object. Fifteen minutes later, the mice were returned to the test environment and monitored for time spent exploring the novel object versus the familiar object. A ratio of novel to familiar interaction was produced where a score of 50% (1:1) would equate to a random (no preference) interaction. The compound (**6s**) significantly increased novel object exploration relative to vehicle treatment in mice dosed with 1, 3, or 10 mg/kg (s.c.) 60 min prior to the acquisition phase ($p < 0.01$, 0.05, and 0.05, respectively, Figure 4). Even though the compound had excellent oral bioavailability, the dosing for this efficacy test was routinely run with subcutaneous administration (s.c.) to stay consistent with the protocol established for other compounds.

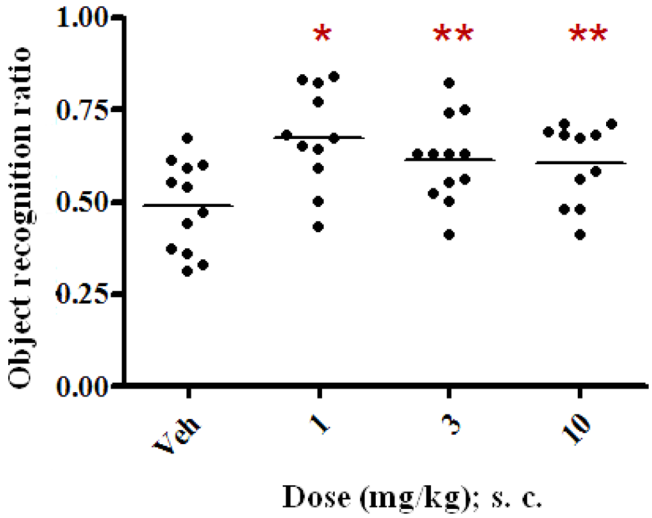


Figure 4. Compound **6s** significantly increased novel object exploration 30 min after acquisition relative to vehicle treatment in mice dosed with 1, 3, and 10 mg/kg 60 min prior to acquisition ($p <$ 0.01, 0.05, and 0.05 respectively). All data are mean ± SD ratio of novel/familiar object interaction. Analysis was performed on manual assessment of interaction scores using ANOVA followed by Newman–Keuls posthoc analysis.

CONCLUSIONS

We have identified a potent and selective series of H_3 antagonists originating from the azaspiro[2.5]octane carboxamide scaffold. Modifications of the spirocyclic core to spirocyclobutyl, 3- and 4-pyrrolidine, and 4-piperidine were largely tolerated. These molecules possess favorable off-target profiles for both secondary pharmacology as well as favorable in vitro DMPK properties. It has been our experience that basic lipophilic amines tend to have multiparameter undesirable SAR (off-target selectivity, microsomal clearance, phospholipidosis, etc.) that often tracks with the primary and desired pharmacology. To optimize to the primary pharmacology, significant chemistry resources are often employed to try to separate the desired functional activity from the undesired activity. We have found that this scaffold, as exemplified by **6s**, lacked many of these undesirable features. This may be due in part to the lack of aromatic rings or perhaps the low logD (e.g.,

Table 7. In Vivo DMPK Brain and Plasma Concentrations

Cpd[a]	plasma (nM)[b]	Brain (nmol/kg)[b]	CSF (nM)[b]	Br/Pl[c]	Cpd[d]	C_{max} (nM)[f]	t_{max} (h)	%F[g]
6s, IV, $n = 4$	180 ± 40	540 ± 120	34 ± 6	3.0 ± 0.7	**6s**, PO[e], $n = 3$	71 ± 9.7	6.0	99 ± 18

[a]Sample taken after 1 h, IV dose (3 μmol/kg). [b]Average plasma concentrations after 1 h with standard devation. Average brain concentration reported with standard deviation. Brain concentrations are not corrected for vascular contamination of ~2–3%. Nonspecific binding during CSF sampling was not evaluated. [c]Brain/plasma ratio. [d]Oral route of administration with n = number of animals, details described in Figure 3. [e]3 μmol/kg, 1:9 ratio of 10% DMA to 90% SBECD, pH 4.73, as vehicle. [f]Maximal concentration of oral dose. [g]Percent oral bioavailability.

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX–XXX

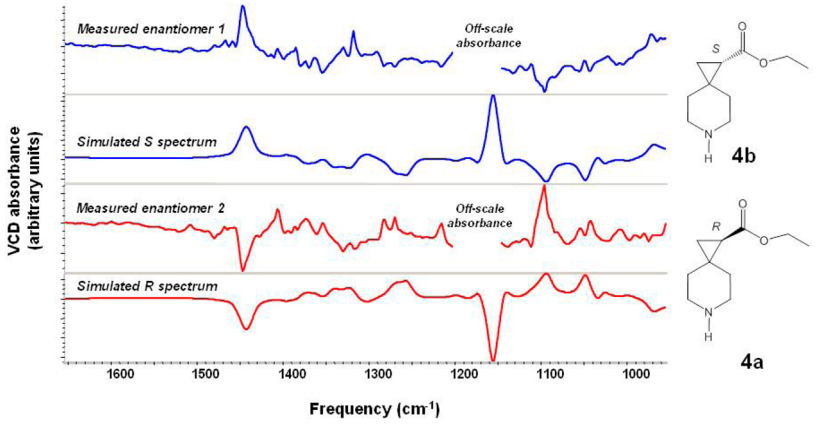


Figure 5. VCD Spectra of the Boltzmann-weighted average of the computed VCD spectra along with the experimental spectra for **4b** (*S*) and **4a** (*R*).

6s = −0.26, measured logD). An exemplar compound from the spirocyclic cyclopropanes, **6s**, demonstrated high brain exposures, high oral bioavailability, and efficacy in a mouse novel object recognition study. The high volumes of distribution and long half-lives of the dibasic compounds provide a significant challenge in both interpretation of PK data and prediction of pharmacodynamic (PD) response. Because the H_3 receptor is hypothesized to be involved in wakefulness, this may present a liability as the compound also possesses high CNS exposure with an exceptionally long half-life. The monobasic compounds provide a very different PK profile with shorter half-lives and lower volumes of distribution that potentially may have better alignment with the intended indication. Compounds from both of these subtypes should provide useful starting points and tools to investigate further the importance of selective H_3 antagonists for drug therapy in a variety of potential disease states.

EXPERIMENTAL SECTION

General Experimental. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance DPX 300 or 500 MHz spectrometer, and the chemical shifts are reported in parts per million (δ) from a tetramethylsilane (TMS) internal standard. The MS detection was performed with a Micromass Platform ZMD or LCZ spectrometers using the indicated ionization method. High-resolution mass spectra were recorded on an Agilent Technologies 6210 time-of-flight LC−MS spectrometer. Chemical purities of final compounds were determined to be >95% as determined by HPLC-MS analysis at UV 220 nm. Unless otherwise indicated, all starting materials were obtained from commercial suppliers. Preparatory HPLC procedure A: Preparatory HPLC was conducted using a 21 × 150 mm, 5 μm Gemini C18 column at pH 10 [0.125 M ammonium bicarbonate] with ACN/H_2O as the mobile phase (solvent flow was 50 mL/min and a gradient from 10% acetonitrile to 93% acetonitrile over 33 min). Preparatory HPLC procedure B: Preparative HPLC was conducted using a long, high pH, shallow gradient method. The mobile phase was as follows: 30−50% B; A: H_2O with 15 mM ammonium bicarbonate and 0.375% NH_4OH v/v, B: CH_3CN. The run was 25 min on XBridge Prep C18 OBD, 30 × 150 mm, Waters reverse-phase column. Preparatory HPLC procedure C: Preparative HPLC was conducted using normal-phase HPLC (mobile phase: CH_2Cl_2/MeOH/NH_4OH, flow: 16 mL/min, 40 g column, gradient running from 100:0:1 to 80:20:1 over 1 h). Analytical HPLC procedure D: Analytical HPLC used a high pH gradient method (mobile phase: 5−95% B; A: H_2O with 10 mM NH_4CO_3 and 0.375% NH_4OH v/v, B: MeOH, 2.25 min run) on X-Bridge C18, 2.1 × 30 mm, 5 μm particle size. Compounds **6a−g**, **6j−m**, **6o−r**, and **6s** have been described in a previous patent application by our group, and experimental details are included below.[34] All final compounds were isolated in >95% purity as judged by LC−MS. Conformations of compounds **6s**, **29f**, **34**, and Bavisant were generated via Monte Carlo searching with the OPLS95 force field in a continuum water model as implemented in MacroModel.[35] Molecular overlays comparing these conformational sets to the small-molecule X-ray structure of Conessine were produced using shape and chemical feature matching with ROCS.[36] Default settings and parameters were used throughout the calculations.

Benzyl 4-Methylenepiperidine-1-carboxylate (2). A solution of methyltriphenylphosphonium bromide (88.2 mmol, 31.5 g) was cooled in 600 mL of THF to 0 °C. To this solution, *n*-BuLi (2.5M, in hexanes, 35.3 mL, 1.2 equiv) was slowly added whereupon a precipitate gradually formed, and the reaction turned reddish-orange in color. The reaction was stirred for 1 h at 0 °C, at which point benzyl 4-oxopiperidine-1-carboxylate **1** was added (73.5 mmol, 17.1 g, in 30 mL THF), and the reaction was stirred for an additional 1 h at 0 °C. The volatiles were removed under reduced pressure, and the reaction mixture was poured onto a pad of SiO_2 and flushed with a 4:1 mixture of hexanes/EtOAc. The product was purified via column chromatography (SiO_2, hexanes/EtOAc) as a clear oil (9.11 g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.23 (t, J = 5.7 Hz, 4H), 3.53 (t, J = 5.9 Hz, 4H), 4.78 (s, 2H), 5.17 (s, 2H), 7.30−7.39 (m, 5H). MS m/z (ES+) $[M + H]^+$ = 232.1.

6-Benzyl 1-Ethyl 6-Azaspiro[2.5]octane-1,6-dicarboxylate (3). Benzyl 4-methylenepiperidine-1-carboxylate **2** (39.6 mmol, 9.17 g) was dissolved in 1300 mL of anhydrous CH_2Cl_2 followed by the addition of CuCN (39.6 mmol, 3.54 g), and the reaction was stirred at room temperature. Ethyl diazoacetate (87.2 mmol, 9.17 mL) was dissolved in 10 mL of CH_2Cl_2 and slowly added (0.2 mL/h, over roughly 100 h). The solution was filtered through SiO_2, and the volatiles were removed. The residue was purified via column chromatography (100% hexanes to 4:1 hexanes/EtOAc) to give the title compound as a clear oil (5.68 g, 45% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (dd, J = 8.0, 4.6 Hz, 1H), 1.20 (t, J = 5.0 Hz, 1H), 1.28 (t, J = 7.1 Hz, 3H), 1.42−1.48 (m, 2H), 1.56−1.61 (m, 1H), 1.69−1.76 (m, 2H), 3.33−3.41 (m, 1H), 3.49−3.65 (m, 3H), 4.16 (q, J = 8.4 Hz, 2H), 5.16 (s, 2H), 7.35−7.38 (m, 5H). MS m/z (ES+) $[M + H]^+$ = 318.2.

(*R*)-6-Benzyl 1-Ethyl 6-Azaspiro[2.5]octane-1,6-dicarboxylate (3a). The material was purified by chiral supercritical fluid chromatography (SFC, AD column with MeOH and 0.1% dimethyl ethlyl amine, isocratic at 35%, 10 mL/min, main eluent CO_2, column temperature 35 °C) to give the title compound with quantitative recovery (t_R = 2.67 min). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 0.94 (dd, J = 7.8, 4.7 Hz, 1H), 1.18 (t, J = 4.8 Hz, 1H), 1.27 (t, J = 7.2 Hz, 3H), 1.44 (br s, 2H), 1.57 (dd, J = 7.8, 5.4 Hz, 1H), 1.73 (br s, 2H), 3.30−3.41 (m, 1H), 3.47−3.66 (m, 3H), 4.14 (q, J = 7.16 Hz, 2H), 5.14 (s, 2H), 7.29−7.35 (m, 1H), 7.35−7.40 (m, 4H). HRMS (TOF) m/z calcd for $C_{18}H_{23}NO_4$ $(M + H)^+$, 318.1699; found, 318.1695. $[\alpha]_D^{20}$ = −70.4 (*c* 2.490, MeOH).

(*S*)-6-Benzyl 1-Ethyl 6-Azaspiro[2.5]octane-1,6-dicarboxylate (3b). The material was purified by chiral SFC chromatography

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

as in **3a** to give the title compound with quantitative recovery (t_R = 2.43 min). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 0.94 (dd, *J* = 8.2, 4.7 Hz, 1H), 1.18 (t, *J* = 4.88 Hz, 1H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.44 (br s, 2H), 1.57 (dd, *J* = 8.2, 5.5 Hz, 1H), 1.72 (br s, 2H), 3.30−3.42 (m, 1H), 3.47−3.66 (m, 3H), 4.14 (q, *J* = 7.3 Hz, 2H), 5.14 (s, 2 H), 7.30−7.35 (m, 1H), 7.35−7.41 (m, 4H). HRMS (TOF) *m*/*z* calcd for $C_{18}H_{23}NO_4$ $(M + H)^+$, 318.1699; found, 318.1699. $[\alpha]_D^{20}$ = +71.7 (*c* 2.306, MeOH).

Ethyl 6-Azaspiro[2.5]octane-1-carboxylate (4). To 6-benzyl 1-ethyl 6-azaspiro[2.5]octane-1,6-dicarboxylate **3** (6.45 g, 20.3 mmol) in ethanol (100 mL) was added 5% Pd/C (0.65 g, 0.30 mmol), and a balloon filled with hydrogen was placed on top of the reaction. The reaction was left to stir at room temperature overnight. The mixture was filtered through Celite, and the solvent was removed under reduced pressure. The resulting oil was placed under high vacuum. The crude material was chromatographed (SiO_2 100 g, 5−10% gradient of 7 N NH_3/MeOH in CH_2Cl_2). The solvent was removed from the combined fractions under reduced pressure to give title compound **4** (1.83g, 44%) as an amber oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (dd, *J* = 8.0, 4.4 Hz, 1H), 1.13 (t, *J* = 4.9 Hz, 1H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.41 (q, *J* = 5.2 Hz, 2H), 1.48−1.52 (m, 2H), 1.66−1.71 (m, 2H), 2.70−2.78 (m, 1H), 2.81−2.85 (m, 1H), 2.89 (t, *J* = 5.4 Hz, 2H), 4.14 (q, *J* = 7.1 Hz, 2H). MS *m*/*z* (ES+) $[M + H]^+$ = 184.

(*R*)-Ethyl 6-Azaspiro[2.5]octane-1-carboxylate (4a). The procedure was identical to **4b** except (*R*)-6-benzyl 1-ethyl 6-azaspiro[2.5]octane-1,6-dicarboxylate **3a** was used to give the title compound in 59% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (dd, 1H), 1.09−1.16 (m, 1H), 1.27 (t, *J* = 7.0 Hz, 3H), 1.34−1.47 (m, 2H), 1.50 (dd, *J* = 7.8, 5.4 Hz, 1H), 1.58 (br s, 1H), 1.61−1.76 (m, 2H), 2.68−2.79 (m, 1H), 2.80−2.87 (m, 1H), 2.89 (t, *J* = 5.8 Hz, 2H), 4.13 (q, *J* = 7.1 Hz, 2H). $[\alpha]_D^{20}$ = −115.1 (*c* 1.285, MeOH). Stereochemistry assigned as (*R*) based on calculated and observed VCD spectra (Figure 5).

(*S*)-Ethyl 6-Azaspiro[2.5]octane-1-carboxylate (4b). A mixture of (*S*)-6-benzyl 1-ethyl 6-azaspiro[2.5]octane-1,6-dicarboxylate **3b** (0.50 g, 1.58 mmol), Pd/C (5%, 8.38 mg, 0.08 mmol), and ethanol (31.5 mL) was shaken overnight in a Parr apparatus under a 20 psi atmosphere of hydrogen. The mixture was filtered over a Celite pad, and the solvent was concentrated. The residue was loaded over a small SiO_2 pad using CH_2Cl_2 and was purified by gravity filtration using CH_2Cl_2 first to elute the yellow colored impurity and then using 5% MeOH, 10% acetone in CH_2Cl_2 to give the title compound as a colorless liquid (0.23 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (dd, *J* = 7.8, 4.3 Hz, 1H), 1.13 (t, *J* = 4.8 Hz, 1H), 1.27 (t, *J* = 6.8 Hz, 3H), 1.44 (dd, *J* = 9.3,4.7 Hz, 2H), 1.51 (dd, *J* = 7.8, 5.4 Hz, 1H), 1.64−1.79 (m, 2H), 2.34 (br s, 1H), 2.70−2.80 (m, 1H), 2.83−2.89 (m, 1H), 2.91 (t, *J* = 5.2 Hz, 2H), 4.14 (q, *J* = 6.7 Hz, 2H). $[\alpha]_D^{20}$ = +106.8 (*c* 1.367, MeOH). Stereochemistry was assigned as (*S*) based on calculated and observed VCD spectra (Figure 5).

Ethyl 6-(Tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octane-1-carboxylate (5). To ethyl 6-azaspiro[2.5]octane-1-carboxylate **4** (1.85 g, 10.0 mmol) in 1,2-dichloroethane (25 mL) was added dihydro-2*H*-pyran-4(3*H*)-one (1.00 mL, 11.0 mmol), and the solution was stirred 25 min. To this was added sodium triacetoxyborohydride (2.50 g, 12.1 mmol), and the reaction was left to stir at room temperature. After 5 h, the reaction was partitioned between EtOAc and $NaHCO_3$ (aq. sat.). The organic layer was removed, and the solvent was evaporated to give a pale oil. The material was chromatographed on SiO_2 (50 g, 7 N NH_3/MeOH in CH_2Cl_2), and the combined purified fractions were removed of solvent to give the title compound (1.82 g, 64%) as a pale oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (dd, *J* = 8.0, 4.5 Hz, 1H), 1.12 (t, *J* = 4.9 Hz, 1H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.47−1.52 (m, 3H), 1.56−1.65 (m, 2H), 1.72−1.78 (m, 4H), 2.39−2.61 (m, 5H), 3.37 (td, *J* = 11.7, 2.1 Hz, 2H), 4.02 (dd, *J* = 11.5, 4.5 Hz, 2H), 4.13 (q, *J* = 7.0 Hz, 2H). *m*/*z* (ES+) $[M + H]^+$ = 268.

(4-Methylpiperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6a). The material was made in an analogous fashion to compound **6d** using 1-methylpiperiazine as the starting material to give the title compound in 45% yield. The material was converted to the citrate salt. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.71−0.75 (m, 1H), 1.26 (t, *J* = 4.7 Hz, 1H), 1.32−1.51 (m, 2H), 1.55−1.82 (m, 8H), 2.28 (s, 3H), 2.38−2.66 (m, 6H), 2.67−2.78 (m, 1H), 3.39 (td, *J* = 11.7, 1.8 Hz, 2H), 3.46−3.60 (m, 2H), 3.66 (t, *J* = 4.7 Hz, 2H), 3.72−3.84 (m, 1H), 4.04 (dd, *J* = 11.1, 4.0 Hz, 2H). HRMS (TOF) *m*/*z* calcd for $C_{18}H_{32}N_3O_2$ $(M + H)^+$, 322.2489; found, 322.2499.

(4-Isopropylpiperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6b). This example was prepared according to the procedure for compound **6d** using commercially available 1-isopropylpiperazine. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71 (dd, *J* = 7.8, 4.4 Hz, 1H), 1.04 (d, *J* = 6.5 Hz, 6H), 1.24 (t, *J* = 4.8 Hz, 1H), 1.36−1.47 (m, 2H), 1.58−1.67 (m, 5H), 1.69−1.76 (m, 2H), 2.39−2.59 (m, 8H), 2.68−2.73 (m, 2H), 3.37 (t, *J* = 11.6 Hz, 2H), 3.51 (m, 1H), 3.63 (s, 2H), 3.76 (m, 1H), 4.02 (dd, *J* = 11.3, 4.3 Hz, 2H). HRMS (TOF) *m*/*z* calcd for $C_{20}H_{35}N_3O_2$ $(M + H)^+$, 350.2802; found, 350.2799.

(4-Cyclopropylpiperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6c). This example was prepared according to the procedure for compound **6d** using commercially available 1-cyclopropylpiperiazine. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.41−0.45 (m, 2H), 0.46−0.50 (m, 2H), 0.69−0.73 (m, 1H), 1.24 (t, *J* = 4.8 Hz, 1H), 1.35−1.46 (m, 2H), 1.57−1.66 (m, 5H), 1.69−1.76 (m, 3H), 2.43−2.72 (m, 9H), 3.37 (t, *J* = 11.2 Hz, 2H), 3.49 (m, 1H), 3.59 (s, 2H), 3.69 (m, 1H), 4.02 (dd, *J* = 11.1, 4.1 Hz, 2H). HRMS (TOF) *m*/*z* calcd for $C_{20}H_{33}N_3O_2$ $(M + H)^+$, 348.2646; found, 348.2654.

(4-Cyclobutylpiperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6d). To ethyl 6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octane-1-carboxylate **5** (1.82 g, 6.81 mmol) were added MeOH (25 mL), 1 N LiOH (7.20 mL, 7.20 mmol), and water (1 mL). The cloudy reaction was left to stir at room temperature. After 5 h, 1 N NaOH (7.2 mL, 7.2 mmol) and 20 mL MeOH were added, and the reaction was stirred at room temperature. After 2 h, solid NaOH (290 mg, 7.3 mmol) was added followed by 5 mL of THF, and the cloudy reaction was stirred overnight. The reaction was then heated to reflux for 2 h, and the solvent was removed under reduced pressure. The gummy residue was dissolved in 15 mL of water, and the pH was adjusted to ∼6 by adding 6 N HCl (3.8 mL). The water was removed under reduced pressure, and residual water was removed from the resulting material by azeotropic distillation with MeOH. The addition and subsequent removal of methanol under reduced pressure was performed several times, and the resulting pale yellow solid was put under high vacuum. To this was added 15 mL of MeOH, and the solution was decanted from the insoluble material. The solvent was removed under reduced pressure to give 2.45 g of crude 6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]-octane-1-carboxylic acid, which was used as is without further purification (∼quan. recovery). MS *m*/*z* (ES+) $[M + H]^+$ = 240. To the intermediate acid (150 mg, 0.63 mmol) were added HBTU (238 mg, 0.63 mmol), DMF (2.0 mL), and DIPEA (0.328 mL, 1.88 mmol), and the reaction was stirred for 5 min. In a separate vial, 1-cyclobutylpiperazine to (160 mg, 0.75 mmol), DMF (1.0 mL), and DIPEA (0.263 mL, 1.50 mmol) were combined. This solution was added to the first reaction, and the yellow solution was stirred at room temperature. After 1.5 h, the reaction was diluted with EtOAc (5 mL), and the organic layer was separated. The aqueous layer was washed three times with CH_2Cl_2 and combined with the first organic layer. The organics were washed three times with saturated $NaHCO_3$ and twice with saturated NaCl, dried over Na_2SO_4, and concentrated under reduced pressure, and the residue was put under high vacuum. The crude material was chromatographed on 20 g silica with 10% (7 N NH_3/MeOH)/CH_2Cl_2, and the combined purified fractions removed of solvent under reduced pressure. The solid was triturated with Et_2O, and the resulting precipitate was collected by filtration to give the title compound (77 mg, 34%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71 (dd, *J* = 7.8, 4.4 Hz, 1H), 1.24 (t, *J* = 4.8 Hz, 1H), 1.35−1.45 (m, 2H), 1.56−1.75 (m, 9H), 1.84−1.92 (m, 2H), 2.01−2.07 (m, 2H), 2.18−2.62 (m, 8H), 2.67−2.76 (m, 2H), 3.37 (t, *J* =

dx.doi.org/10.1021/jm4014828 | *J. Med. Chem.* XXXX, XXX, XXX−XXX

11.2 Hz, 2H), 3.52 (m, 1H), 3.64 (t, J = 4.7 Hz, 2H), 3.75 (m, 1H), 4.02 (dd, J = 11.2, 4.1 Hz, 2H). HRMS (TOF) m/z calcd for $C_{21}H_{35}N_3O_2$ $[M + H]^+$, 362.2802; found, 362.2808.

(4-Cyclopentylpiperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6e). This example was prepared in an analogous fashion to compound **6d** using 1-cyclopentylpiperazine dihydrochloride.[24] ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71 (dd, J = 7.8, 4.4 Hz, 1H), 1.24 (t, J = 4.8 Hz, 1H), 1.37−1.48 (m, 3H), 1.55−1.76 (m, 12H), 1.83−1.88 (m, 2H), 2.36−2.62 (m, 9H), 2.70−2.74 (m, 1H), 3.37 (t, J = 11.0 Hz, 2H), 3.52 (m, 1H), 3.65 (t, J = 4.8 Hz, 2H), 3.76 (m, 1H), 4.03 (dd, J = 11.3, 4.1 Hz, 2H). HRMS (TOF) m/z calcd for $C_{22}H_{37}N_3O_2$ $(M + H)^+$, 376.2959; found, 376.2957.

(4-Cyclohexylpiperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6f). Piperazin-1-yl(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone **11** (0.36 mmol, 0.19 g), cyclohexanone (1.08 mmol, 0.11 mL), 3 drops of glacial acetic acid, and polystyrylmethyl trimethylammonium cyanoborohydride (0.70 mmol, 0.19 g, 4.1 mmol/g of resin) were slurried in 20 mL of CH_2Cl_2 and stirred overnight at room temperature. The solution was filtered through a nylon filter, and the volatiles were removed. The residue was purified via column chromatography, and the product was eluted with CH_2Cl_2/CH_2Cl_2 containing 10% (2 M NH_3/MeOH) on SiO_2 over 30 min. The product was isolated as a white solid (92 mg, 66% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.61−0.65 (m, 1H), 0.95−0.98 (m, 1H), 1.07−1.22 (m, 6H), 1.30−1.55 (m, 6H), 1.55−1.82 (m, 6H), 2.25−2.58 (m, 11H), 3.22−3.29 (m, 2H), 3.58−3.64 (m, 3H), 3.84−3.89 (m, 2H). HRMS (TOF) m/z calcd for $C_{23}H_{40}N_3O_2$ $(M + H)^+$, 390.3115; found, 390.3115.

(4-Cycloheptylpiperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6g). This example was prepared according to the procedure for compound **6d** using commercially available 1-cycloheptylpiperazine. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.70 (dd, J = 7.8, 4.4 Hz, 1H), 1.24 (t, J = 4.8 Hz, 1H), 1.37−1.82 (m, 21H), 2.39−2.60 (m, 9H), 2.67−2.71 (m, 1H), 3.37 (t, J = 11.7 Hz, 2H), 3.46 (m, 1H), 3.65 (s, 2H), 3.76 (m, 1H), 4.02 (dd, J = 11.2, 4.1 Hz, 2H). HRMS (TOF) m/z calcd for $C_{24}H_{41}N_3O_2$ $(M + H)^+$, 404.3272; found, 404.3278.

(4-Cyclobutyl-1,4-diazepan-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6h). To a solution of 6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octane-1-carboxylic acid (0.10 g, 0.42 mmol) in DMF (2 mL) were added HOBT (0.096 g, 0.63 mmol), 1-cyclobutyl-1,4-diazepane dihydrochloride (0.11 g, 0.50 mmol), DIPEA (0.15 mL, 0.84 mmol), and EDCI (0.12 g, 0.63 mmol) followed by DMF (2.0 mL). The reaction was then stirred overnight at room temperature. The solvent was removed, and residual material was purified with preparatory HPLC procedure A to provide the title compound as a white solid (0.15, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.70 (dd, J = 10.0, 5.0 Hz, 1H), 1.25−1.27 (m, 2H), 1.47−1.48 (m, 1H), 1.58−1.63 (m, 9H), 1.74−1.92 (m, 6H), 2.00−2.03 (m, 2H), 2.27−2.31 (m, 2H), 2.46−2.49 (m, 2H), 2.61−2.63 (m, 2H), 2.72−2.74 (m, 1H), 2.84 (quin, J = 10.0 Hz, 1H), 3.37 (t, J = 10.0 Hz, 2H), 3.75−3.57 (m, 4H), 4.02 (dd, J = 10.0 Hz, 5.0 Hz, 2H). HRMS (TOF) m/z calcd for $C_{22}H_{37}N_3O_2$ $(M + H)^+$, 376.2958; found, 376.2963.

(4-(Pyridin-4-yl)piperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6i). The material was prepared in a similar fashion to compound **6d** beginning with 1-(pyridin-4-yl)piperazine (0.09 g, 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (dd, J = 8.0, 4.8 Hz, 1H), 1.29 (t, J = 4.7 Hz, 1H), 1.32−1.42 (m, 1H), 1.42−1.53 (m, 1H), 1.53−1.74 (m, 4H), 1.74−1.95 (m, 3H), 2.37−2.60 (m, 3H), 2.60−2.69 (m, 1H), 2.69−2.81 (m, 1H), 3.26−3.51 (m, 6H), 3.67−3.91 (m, 4H), 4.03 (dd, J = 11.1, 4.1 Hz, 2H), 6.67 (d, J = 6.2 Hz, 2H), 8.33 (d, J = 5.1 Hz, 2H). HRMS (TOF) m/z calcd for $C_{22}H_{32}N_4O_2$ $(M + H)^+$, 385.2598; found, 385.2595.

(6-Cyclobutyl-6-azaspiro[2.5]octan-1-yl)(4-(tetrahydro-2*H*-pyran-4-yl)piperazin-1-yl)methanone (6j). To benzyl 1-(4-(tetrahydro-2*H*-pyran-4-yl)piperazine-1-carbonyl)-6-azaspiro[2.5]-octane-6-carboxylate **12** (0.10 g, 0.23 mmol) were added MeOH (1.0 mL), Pd/C (4.82 mg, 4.53 μmol), oven-dried 3 Å powdered molecular sieves, and cyclobutanone (0.04 mL, 0.45 mmol), and a balloon filled with H_2 (excess) was fixed atop the reaction. This was stirred overnight. The reaction was filtered, the solvent was removed, and the clear gum was put under high vacuum. The material was purified by reverse-phase HPLC using procedure A. The fractions were collected, and the solvent was removed, and the residue was dried under high vacuum to give the title compound (0.08 g, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71 (m, 1H), 0.88 (M, 1H), 1.24 (t, J = 4.5 Hz, 1H), 1.39−1.49 (m, 2H), 1.60−1.75 (m, 7H), 1.86−1.90 (m, 2H), 2.01−2.09 (m, 2H), 2.13−2.29 (m, 2H), 2.58−2.62 (m, 5H), 2.63−2.67 (m, 2H), 2.69−2.72 (m, 2H), 2.73−2.86 (m, 1H), 3.35 (t, J = 11.5 Hz, 2H), 3.60−3.63 (m, 2H), 3.77−3.79 (m, 1H), 4.00 (d, J = 11.5 Hz, 2H). HRMS (TOF) m/z calcd for $C_{21}H_{36}N_3O_2$ $(M + H)^+$, 362.2802; found, 362.2801.

(4-Cyclohexylpiperazin-1-yl)(6-azaspiro[2.5]octan-1-yl)-methanone (6k). To benzyl 1-(4-cyclohexylpiperazine-1-carbonyl)-6-azaspiro[2.5]octane-6-carboxylate (3.21 g, 7.30 mmol) in MeOH (25 mL) was added 5% Pd/C (0.31 g, 0.15 mmol), and a balloon of hydrogen was fixed atop the reaction. The reaction was stirred at room temperature. After 2 h, the reaction was filtered through Celite and concentrated. The filtrate was removed of solvent, and the residual white solid was put under high vacuum to give the title compound (2.12 g, 95%). The material was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71−0.75 (m, 1H), 1.10−1.40 (m, 8H), 1.56−1.58 (m, 5H), 1.76−1.85 (m, 4H), 2.26−2.29 (m, 1H), 2.43−2.46 (m, 1H), 2.52−2.57 (m, 2H). 2.64−2.66 (m, 1H), 2.74−2.79 (m, 2H), 2.80−2.94 (m, 2H), 3.46−3.49 (m, 1H), 3.60−3.65 (m, 2H), 3.75−3.78 (m, 1H). HRMS (TOF) m/z calcd for $C_{18}H_{31}N_3O$ $(M + H)^+$, 306.2539; found, 306.2542.

(6-Cyclohexyl-6-azaspiro[2.5]octan-1-yl)(4-cyclohexylpiperazin-1-yl)methanone (6l). The title compound was prepared in analogous fashion to compound **6b** (0.03 g, 25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.68−0.69 (m, 1H), 1.23−1.30 (m, 10H), 1.39−1.44 (m, 2H), 1.57−1.60 (m, 6H), 1.81−1.84 (m, 8H), 2.43−2.48 (m, 2H), 2.55−2.59 (m, 6H), 2.64−2.66 (m, 2H), 3.44−3.47 (m, 1H), 3.60−3.63 (m, 2H), 3.73−3.75 (m, 1H). HRMS (TOF) m/z calcd for $C_{24}H_{41}N_3O$ $(M + H)^+$, 388.3322; found, 388.3328.

(4-Cyclohexylpiperazin-1-yl)(6-isopropyl-6-azaspiro[2.5]-octan-1-yl)methanone (6m). The title compound was prepared in analogous fashion to compound **6b** (0.03 g, 26%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.75−0.72 (m, 1H), 1.02−1.07 (m, 6H), 1.22−1.26 (m, 6H), 1.42−1.45 (m, 2H), 1.60−1.63 (m, 2H), 1.78−1.84 (m, 6H), 2.24−2.27 (m, 1H), 2.36−2.39 (m, 1H), 2.48−2.52 (m, 5H), 2.69−2.71 (m, 2H), 2.78 (quin, J = 10.5 Hz, 1H), 3.47−3.49 (m, 1H), 3.58−3.63 (m, 2H), 3.76−3.79 (m, 1H). HRMS (TOF) m/z calcd for $C_{21}H_{37}N_3O$ $(M + H)^+$, 348.3009; found, 348.3010.

(4-Cyclohexylpiperazin-1-yl)(6-phenyl-6-azaspiro[2.5]octan-1-yl)methanone (6n). To (4-cyclohexylpiperazin-1-yl)(6-azaspiro[2.5]octan-1-yl)methanone **6k** (0.10 g, 0.33 mmol) in toluene (1.5 mL) were added bromobenzene (0.05 mL, 0.43 mmol), *trans*-dichlorobis(tri-*o*-tolyl-phosphine)palladium(II) (0.03 g, 0.03 mmol), and LiHMDS (1 M in toluene, 0.39 mL, 0.39 mmol). The reaction was heated at 100 °C for 1 h. The reaction was quenched with 2 mL of EtOAc, and the precipitate was filtered off and put under high vacuum overnight. The sample was chromatographed (SiO_2, CH_2Cl_2 to 5% (7 N NH_3)/MeOH). The fractions were collected and dried to yield a crude solid. The HCl salt was made by dissolving in 1 N HCl/MeOH, and the solvent was removed. The solids were triturated with Et_2O, filtered, and put under high vacuum. The compound was further purified by dissolving in MeOH and passing through 4 g Isolute NH_2 column with MeOH. The product was removed of solvent, dissolved in ACN, and filtered through a 0.45 μM frit. After sitting at room temperature for a while, the material crystallized out, filtered, and dried under high vacuum at 50 °C for 5 h to give the title compound as a white solid (0.015 g, 12%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71 (dd, J = 7.8, 4.4 Hz, 1H), 1.10−1.24 (m, 5H), 1.32 (t, J = 4.5 Hz, 1H), 1.50−1.65 (m, 3H), 1.74−1.82 (m, 6H), 2.76 (app t, J = 11 Hz, 1H), 2.46−2.48 (m, 2H), 2.56−2.61 (m, 2H), 2.56−2.61 (m, 2H), 3.07−3.10 (m, 1H), 3.17−3.27 (m, 1H), 3.29−3.30 (m, 1H), 3.48−3.50 (m, 1H), 3.62−3.65 (m, 2H), 3.73−3.76 (m, 1H), 6.84 (t, J = 7 Hz, 1H), 6.95

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

(d, J = 8 Hz, 2H), 7.23 (m, 2H). HRMS (TOF) m/z calcd for $C_{24}H_{35}N_3O$ $(M + H)^+$, 382.2852; found, 382.2850.

(6-Benzyl-6-azaspiro[2.5]octan-1-yl)(4-cyclohexylpiperazin-1-yl)methanone (6o). The title compound was prepared in analogous fashion to compound **6b** (0.07 g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83−0.84 (m, 1H), 1.26−1.32 (m, 6H), 1.48−1.50 (m, 4H), 1.58−1.61 (m, 3H), 1.75−1.86 (m, 4H), 2.24−2.31 (m, 2H), 2.52−2.64 (m, 6H), 3.48−3.50 (m, 1H), 3.52 (s, 2H), 3.60−3.61 (m, 2H), 3.70−3.74 (m, 1H), 7.22−7.30 (m, 5H). HRMS (TOF) m/z calcd for $C_{25}H_{37}N_3O$ $(M + H)^+$, 396.3009; found, 396.3004.

(4-Cyclohexylpiperazin-1-yl)(6-phenethyl-6-azaspiro[2.5]-octan-1-yl)methanone (6p). To (4-cyclohexylpiperazin-1-yl)(6-azaspiro[2.5]octan-1-yl)methanone **6k** (0.10 g, 0.33 mmol) was added 1,2-dichloroethane (2 mL) and 2-phenylacetaldehyde (37 μL, 0.33 mmol). The reaction was stirred 30 min, and sodium triacetoxyborohydride (0.76 g, 0.36 mmol) was added. The reaction was stirred at room temperature overnight. To the reaction mixture was added 1.5 mL of 1 N NaOH, and the mixture was stirred for 20 min. The aqueous layer was removed, to this were added Et_2O and saturated NaCl, and the mixture was stirred for 5 min. The aqueous layer was removed, the organic layers were dried over Na_2SO_4, the solvent was removed, and the material was put under high vacuum at 50 °C overnight. The material was purified on 12 g silica gel eluting with CH_2Cl_2 to 10% (7 N NH_3/MeOH)/CH_2Cl_2. The product was removed of solvent and put under high vacuum. The material was dissolved in hot hexanes and cooled in a freezer to give a white solid that was collected to give the final compound. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82−0.85 (m, 1H), 1.25−1.31 (m, 6H), 1.57−1.58 (m, 2H), 1.63−1.67 (m, 4H), 1.79−1.85 (m, 4H), 2.24−2.27 (m, 1H), 2.58−2.69 (m, 10H), 2.80−2.84 (m, 2H), 3.51−3.57 (m, 1H), 3.62−3.64 (m, 2H), 3.79−3.81 (m, 1H), 7.18−7.21 (m, 3H), 7.27−7.30 (m, 2H), HRMS (TOF) m/z calcd for $C_{26}H_{39}N_3O$ $(M + H)^+$, 410.3165; found, 410.3169.

(4-Cyclohexylpiperazin-1-yl)(6-(pyridin-4-yl)-6-azaspiro[2.5]-octan-1-yl)methanone (6q). To (4-cyclohexylpiperazin-1-yl)(6-azaspiro[2.5]octan-1-yl)methanone **6k** (0.10 g, 0.33 mmol) were added 4-chloropyridine hydrochloride (0.05 g, 0.33 mmol), K_2CO_3 (0.11 mg, 0.82 mmol), and DMSO (2 mL). The reaction was sealed and placed in a 90 °C bath overnight. The reaction was diluted with EtOAc/NaCl (sat.) and washed with NaCl (sat., 2×), dried over Na_2SO_4, and stripped to give an orange oil. The material was purified by chromatography (SiO_2, CH_2Cl_2 to 10% (7 N NH_3/MeOH)/CH_2Cl_2). The product was removed of solvent and put under high vacuum at 50 °C overnight to give the title compound as a white solid (0.02 g, 26%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71 (dd, J = 7.8, 4.4 Hz, 1H), 1.46−1.30 (m, 6H), 1.46−1.48 (m, 1H), 1.50−1.55 (m, 1H), 1.54−1.58 (m, 2H), 1.63−1.69 (m, 2H), 1.74−1.81 (m, 4H), 2.24−2.27 (m, 1H), 2.47−2.57 (m, 2H), 2.57−2.70 (m, 2H), 3.20−3.25 (m, 1H), 3.28−3.32 (m, 1H), 3.33−3.43 (m, 1H), 3.50−3.55 (m, 2H), 3.55−3.60 (m, 2H), 3.65−3.72 (m, 1H), 6.60 (d, J = 5.5 Hz, 2H), 8.26 (d, J = 5.5 Hz, 2H). HRMS (TOF) m/z calcd for $C_{23}H_{34}N_4O$ $(M + H)^+$, 383.2805; found, 383.2805.

(*R*)-(4-Isopropylpiperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6r). The procedure was the same as **6s** except (*R*)-benzyl 1-(4-isopropylpiperazine-1-carbonyl)-6-azaspiro[2.5]octane-6-carboxylate was used to give the title compound (2.46 g, 45%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 0.79 (dd, 1H), 1.09 (d, J = 6.6 Hz, 6H), 1.14 (t, J = 4.8 Hz, 1H), 1.40−1.51 (m, 1H), 1.51−1.74 (m,5H), 1.77−1.89 (m, 3H), 2.46 (ddd, J = 11.3, 7.8, 3.1 Hz, 1H), 2.50−2.62 (m, 5H), 2.62−2.81 (m, 4H), 3.40 (t, J = 11.9 Hz, 2H), 3.50 (ddd, J = 13, 7.7, 3.3 Hz, 1H), 3.70 (dt, J = 6.6, 3.3 Hz, 1H), 3.74 (t, J = 5.0 Hz, 2H), 3.99 (dd, J = 11.0, 4.5 Hz, 2H). HRMS (TOF) m/z calcd for $C_{20}H_{35}N_3O_2$ $(M + H)^+$, 350.2802; found, 350.2796. $[\alpha]_D^{20}$ = +32.4 (c 1.766, MeOH).

(*S*)-(4-Isopropylpiperazin-1-yl)(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octan-1-yl)methanone (6s). Pd/C (5%, 0.015 g, 0.14 mmol) was added to a solution of (*S*)-benzyl 1-(4-isopropylpiperazine-1-carbonyl)-6-azaspiro[2.5]octane-6-carboxylate (2.80 g, 7.01 mmol) and dihydro-2*H*-pyran-4(3*H*)-one (1.05 g, 10.51 mmol) in methanol (28 mL). The reaction mixture was shaken overnight in a Parr apparatus under 30 psi of a H_2 atmosphere. The reaction mixture was filtered over a Celite bed, and the solvent was concentrated. The product was purified by chromatography (SiO_2, using 5% of (7 N NH_3 in MeOH) in CH_2Cl_2 as the eluent) to provide the title compound as white solid (1.68 g, 68.6%). ^{1}H NMR (400 MHz, CD_3OD) δ 0.79 (dd, 1H), 1.09 (d, J = 6.2 Hz, 6H), 1.14 (t, J = 4.8 Hz, 1H), 1.40−1.51 (m, 1H), 1.51−1.74 (m, 5H), 1.78−1.89 (m, 3H), 2.42−2.50 (m, 1H), 2.50−2.62 (m, 5H), 2.63−2.79 (m, 4H), 3.40 (t, J = 11.9 Hz, 2H), 3.50 (ddd, J = 13, 7.6, 3.1 Hz, 1H), 3.70 (ddd, J = 6.4, 3.5, 3.3 Hz, 1H), 3.74 (t, J = 5.0 Hz, 2H), 3.99 (dd, J = 11.5, 4.4 Hz, 2H). HRMS (TOF) m/z calcd for $C_{20}H_{35}N_3O_2$ $(M + H)^+$, 350.2802; found, 350.2796. $[\alpha]_D^{20}$ = −31.1 (c 1.766, MeOH).

(4-Cyclobutylpiperazin-1-yl)(6-cyclohexylspiro[2.5]octan-1-yl)methanone (6t). 6-Cyclohexylspiro[2.5]octane-1-carboxylic acid was prepared by hydrolysis from **17** via a route analogous to preparation of compound **7**. To 6-cyclohexylspiro[2.5]octane-1-carboxylic acid (0.20 g, 0.85 mmol) and *O*-benzotriazol-1-yl-tetramethyluronium hexafluorophosphate (0.32 g, 0.85 mmol) were added DMF (10 mL) and DIPEA (0.74 mL, 4.23 mmol). The mixture was stirred for 5 min, and solid 1-cyclobutylpiperazine·2HCl (0.27 g, 1.27 mmol) was added. The reaction was stirred for 15 min, and the solvent was removed. The material was purified by preparatory HPLC procedure A to give the title compound (0.30 g, 69%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.60−0.62 (m, 1H), 0.82−0.85 (m, 1H), 1.09−1.19 (m, 10H), 1.43−1.49 (m, 3H), 1.67−1.72 (m, 10H), 1.80−1.82 (m, 2H), 1.94−1.96 (m, 2H), 2.20−2.24 (m, 1H), 2.26−2.30 (m, 1H), 2.30−2.45 (m, 2H), 2.68−2.70 (m, 1H), 3.42−3.47 (m, 1H), 3.50−3.55 (m, 1H), 3.65−3.68 (m, 1H), 3.72−3.75 (m, 1H). HRMS (TOF) m/z calcd for $C_{23}H_{39}N_2O$ $(M + H)^+$, 359.3057; found, 359.3072.

6-(Benzyloxycarbonyl)-6-azaspiro[2.5]octane-1-carboxylic acid (7). 6-Benzyl 1-ethyl 6-azaspiro[2.5]octane-1,6-dicarboxylate **3** (4.06 mmol, 1.29 g) was dissolved in MeOH (8 mL), THF (8 mL), and H_2O (8 mL). To this was added LiOH (8.13 mmol, 0.33 g), and the reaction was stirred overnight at room temperature. After the reaction was complete, it was acidified to pH ∼1 with 1 N HCl and extracted with EtOAc. The organics were dried over $MgSO_4$ and filtered, and the residual material was dried under vacuum to give the title compound as a white solid (1.10 g, 94% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83−0.94 (m, 1H), 1.09−1.15 (m, 1H), 1.30−1.40 (m, 2H), 1.46−1.52 (m, 1H), 1.61−1.70 (m, 2H), 3.23−3.54 (m, 4H), 5.06 (s, 2H), 7.20−7.28 (m, 5H). MS m/z (ES+) $[M + H]^+$ = 290.1.

(*R*)-6-Benzyl 1-Ethyl 6-Azaspiro[2.5]octane-1,6-dicarboxylate (7a). The procedure was identical to **7b** except (*R*)-6-benzyl 1-ethyl 6-azaspiro[2.5]octane-1,6-dicarboxylate **3a** was used to give the title compound as a colorless oil (2.77 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (dd, 1H), 1.18−1.26 (m, 1H), 1.46 (br s, 2H), 1.59 (dd, J = 7.81, 5.47 Hz, 1H), 1.77 (br s, 2H), 3.41−3.50 (m, 1H), 3.50−3.61 (m, 3H), 5.14 (s, 2H), 7.30−7.34 (m, 1H), 7.35−7.40 (m, 4 H).

(*S*)-6-(Benzyloxycarbonyl)-6-azaspiro[2.5]octane-1-carboxylic Acid (7b). NaOH (9.58 mL, 19.16 mmol) was added to a solution of (*S*)-6-benzyl 1-ethyl 6-azaspiro[2.5]octane-1,6-dicarboxylate (3.04 g, 9.58 mmol) in methanol (30 mL) and water (10 mL). The reaction mixture was heated and stirred until all of the starting material was consumed, as indicated by TLC. The solvent was concentrated, and water was added. The solution was acidified to pH ∼2 using a 2 M HCl solution. The product was extracted with EtOAc (3 × 50 mL). The organics were combined, dried over anhydrous Na_2SO_4, and concentrated to provide the title compound (2.80 g, 101%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (dd, 1H), 1.17−1.25 (m, 1H), 1.45 (br s, 2H), 1.58 (dd, J = 7.81, 5.47 Hz, 1 H), 1.76 (br s, 2H), 3.38−3.48 (m, 1 H), 3.48−3.61 (m, 3H), 5.14 (s, 2H), 7.29−7.34 (m, 1H), 7.34−7.40 (m, 4H). MS (ES+) m/z $[M + H]^+$ = 290.1. $[\alpha]_D^{20}$ = +69.2 (c 2.349, MeOH).

Benzyl 1-(4-(*tert*-Butoxycarbonyl)piperazine-1-carbonyl)-6-azaspiro[2.5]octane-6-carboxylate (8). 6-(Benzyloxycarbonyl)-6-azaspiro[2.5]octane-1-carboxylic acid **7** (1.90 mmol, 0.55 g), *O*-(7-azabenzotriazole-1-yl)-*N*, *N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (3.80 mmol, 1.44 g), DIPEA (3.80 mmol, 0.66 mL), and piperazine-1-carboxylic acid *tert*-butyl ester (2.85 mmol, 0.53 g) were

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

combined in 50 mL of anhydrous acetonitrile and stirred at room temperature overnight. The reaction was diluted with EtOAc and washed with 100 mL of 1 N NaOH. The organics were then dried over $MgSO_4$ and filtered through a silica gel plug, and the volatiles were removed under reduced pressure. The residue was purified by column chromatography (SiO_2, hexanes/ethyl acetate gradient (100% hexanes to 50% hexanes over 30 min)) to produce the title compound (0.60 g, 69% yield) as a tan solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.74 (dd, J = 7.7, 4.6 Hz, 1H), 1.19−1.27 (m, 2H), 1.40 (s, 9H), 1.45−1.62 (m, 4H), 3.18−3.69 (m, 12H), 5.06 (s, 2H), 7.34 (s, 5H). MS (ES+) m/z $[M + Na]^+$ = 480.2.

***tert*-Butyl 4-(6-Azaspiro[2.5]octane-1-carbonyl)piperazine-1-carboxylate (9).** Benzyl 1-(4-(*tert*-butoxycarbonyl)piperazine-1-carbonyl)-6-azaspiro[2.5]octane-6-carboxylate (1.31 mmol, 0.60 g) was dissolved in 100 mL of EtOH and purged with N_2 for 15 min. This was followed by the addition of 3 drops of glacial acetic acid and 5% Pd/C (0.06 g, 10% by weight of piperazine), and the reaction was placed under a balloon of H_2. The reaction was then stirred overnight at room temperature. The reaction was filtered through a Celite plug, and the volatiles were removed under reduced pressure. The residue was purified via column chromatography and eluted with a CH_2Cl_2/CH_2Cl_2 containing 10% (2 M NH_3/MeOH) gradient over 30 min to give the title compound as clear oil (0.42 g, 99% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 0.79 (dd, J = 7.8, 4.3 Hz, 1H), 1.15 (t, J = 4.8 Hz, 1H), 1.32−1.44 (m, 2H), 1.47 (s, 9H), 1.50−1.68 (m, 2H), 1.83 (dd, J = 7.8, 5.3 Hz, 1H), 2.72−2.76 (m, 2H), 2.83−2.89 (m, 2H), 3.37−3.78 (m, 8H). MS (ES+) m/z $[M + H]^+$ = 324.3.

***tert*-Butyl 4-(6-(Tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]-octane-1-carbonyl)piperazine-1-carboxylate (10).** *tert*-Butyl 4-(6-azaspiro[2.5]octane-1-carbonyl)piperazine-1-carboxylate **9** (1.30 mmol, 0.42 g), dihydro-2*H*-pyran-4(3*H*)-one (1.95 mmol, 0.13 mL), 3 drops of glacial acetic acid, and $NaBH_3CN$ (2.92 mmol, 0.183 g) were combined in 60 mL of anhydrous EtOH and stirred for 12 h at room temperature followed by heating to reflux for 6 h. The reaction was cooled to room temperature, diluted with EtOAc, and washed with 50 mL of 1 N NaOH. The organics were dried over Na_2SO_4 and filtered through a glass filter frit, and the volatiles were removed under reduced pressure. The residue was purified by column chromatography (SiO_2, CH_2Cl_2/CH_2Cl_2 containing 10% (2 M NH_3/MeOH) gradient over 30 min) to produce the title compound (0.44 g, 83% yield) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (dd, J = 8.0, 4.6 Hz, 2H), 1.23 (t, J = 4.8 Hz, 2H), 1.44 (s, 9H), 1.39−1.55 (m, 4H), 1.55−1.75 (m, 4H), 1.80−1.97 (m, 2H), 2.72−2.97 (m, 5H), 3.34−3.58 (m, 6H), 4.03 (dd, J = 11.4, 3.5 Hz, 2H). MS (ES+) m/z $[M + H]^+$ = 408.3.

Piperazin-1-yl(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]-octan-1-yl)methanone (11). *tert*-Butyl-4-(6-(tetrahydro-2*H*-pyran-4-yl)-6-azaspiro[2.5]octane-1-carbonyl)piperazine-1-carboxylate **10** (1.08 mmol, 0.44 g) was dissolved in 25 mL of CH_2Cl_2 followed by the addition of 5 mL of TFA, and the reaction was stirred at room temperature for 1.5 h. The volatiles were removed under reduced pressure, and the product was dried overnight under high vacuum to give the title compound (0.57 g as a tan TFA salt, 99% yield), which was used without further purification. ^{1}H NMR (300 MHz, CD_3OD) δ 0.97−1.10 (m, 2H), 1.24−1.65 (m, 4H), 1.71−1.89 (m, 3H), 1.95−2.15 (m, 4H), 3.06−3.49 (m, 5H), 3.55−3.69 (m, 6H), 3.71−4.13 (m, 4H), MS (ES+) m/z $[M + H]^+$ = 308.3.

Benzyl 1-(4-(Tetrahydro-2*H*-pyran-4-yl)piperazine-1-carbonyl)-6-azaspiro[2.5]octane-6-carboxylate (12). To 6-(benzyloxycarbonyl)-6-azaspiro[2.5]octane-1-carboxylic acid **7** (1.95 g, 6.74 mmol) were added DMF (50 mL), *O*-benzotriazol-1-yl-tetramethyluronium hexafluorophosphate (2.56 g, 6.74 mmol), and DIPEA (5.89 mL, 33.7 mmol). After stirring for 5 min, to this was added 1-(tetrahydro-2*H*-pyran-4-yl)piperazine dihydrochloride (1.72 g, 7.08 mmol). The reaction was stirred for 1 h, and the solvent was removed. The material was partitioned between EtOAc/$NaHCO_3$. The organic layer was washed with $NaHCO_3$ (2×) and NaCl (1×) and dried over Na_2SO_4, and the solvent was removed. The material was chromatographed (330 g SiO_2, with gradient running from pure CH_2Cl_2 to 5% MeOH). The material was converted to the TFA salt and placed under high vacuum at 50 °C overnight to give the title compound as a white solid (0.44 g, 11%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.02 (br s, 1H), 1.27−1.33 (m, 3H), 1.59−1.61 (m, 1H), 1.70−1.71 (m, 1H), 1.82−1.84 (m, 1H), 1.99−2.25 (m, 4H), 2.94 (br s, 1H), 3.22 (br s 1H), 3.22−3.25 (m, 3H), 3.54−3.57 (m, 3H), 3.63−3.72 (m, 3H), 3.92−4.10 (m, 4H), 4.29−4.41 (m, 3H), 4.83 (br s, 1H), 5.15−5.17 (m, 2H), 7.33−7.48 (m, 5H). MS (ES+) m/z $[M + H]^+$ = 442.2.

Benzyl 1-(4-Cyclohexylpiperazine-1-carbonyl)-6-azaspiro[2.5]octane-6-carboxylate (13). To 6-(benzyloxycarbonyl)-6-azaspiro[2.5]octane-1-carboxylic acid **7** (2.99 g, 10.3 mmol) were added DMF (40 mL), HBTU (3.92 g, 10.3 mmol), and DIPEA (5.41 mL, 31.0 mmol), and the mixture was stirred for 5 min. To this was added 1-cyclohexylpiperazine (1.91 g, 11.4 mmol), and the reaction was stirred for 2 h. The DMF was partially removed under vacuum, and the remaining material was partitioned between EtOAc/$NaHCO_3$. The organic layer was washed with $NaHCO_3$ (aq. sat.) and NaCl (aq. sat.) and dried over Na_2SO_4. The solvent was removed, and the material was chromatographed (SiO_2, 2.5% MeOH/CH_2Cl_2). Fractions were combined, and the solvent was evaporated to give the title compound (3.21 g 70%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 086−0.87 (m, 1H), 1.10−1.30 (m, 8H), 1.40−1.43 (m, 1H), 1.59−1.64 (m, 4H), 1.78−1.83 (m, 4H), 2.25−2.29 (m, 1H), 2.37 (s, 1H), 2.45−2.49 (m, 2H), 2.64−2.65 (m, 1H), 3.28−3.30 (m, 1H), 3.30−3.38 (m, 1H), 3.41−3.48 (m, 1H), 3.60−3.69 (m, 2H), 3.69−3.72 (m, 2H), 5.13 (s, 2H), 7.29−7.32 (m, 1H), 7.32−7.36 (m, 4H).

(*R*)-Benzyl 1-(4-Isopropylpiperazine-1-carbonyl)-6-azaspiro[2.5]octane-6-carboxylate (14a). The procedure was identical to **14b** except (*R*)-6-(benzyloxycarbonyl)-6-azaspiro[2.5]-octane-1-carboxylic acid **7a** was used to give the title compound as a white solid (3.7 g, 100%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75 (br s, 1H), 0.87−1.13 (m, 7H), 1.20−1.35 (m, 2H), 1.39 (br s, 2H), 1.50 (br s, 1H), 1.90 (br s, 1H), 2.37 (br s, 2H), 3.20−3.42 (m, 6H), 3.50 (br s, 4H), 5.07 (s, 2H), 7.27−7.42 (m, 5H). MS (ES+) m/z $[M + H]^+$ = 400.3. $[\alpha]_D^{20}$ = +13.1 (c 2.034, MeOH).

(*S*)-Benzyl 1-(4-Isopropylpiperazine-1-carbonyl)-6-azaspiro[2.5]octane-6-carboxylate (14b). HBTU (3.98 g, 10.5 mmol) was added to a solution of (*S*)-6-(benzyloxycarbonyl)-6-azaspiro[2.5]octane-1-carboxylic acid **7b** (2.76 g, 9.54 mmol) in DMF (65 mL). Upon complete dilution, DIEA (5.00 mL, 28.6 mmol) was added, and the reaction mixture was stirred for 5 min. 1-(2-Propyl)-piperazine (1.85 g, 14.3 mmol) was then added, the reaction mixture was stirred for 1 h, and the solvent was concentrated. The residue was recovered in EtOAc (200 mL) and washed with saturated $NaHCO_3$ solution (3 × 50 mL) and brine. The solution was dried over anhydrous Na_2SO_4 and concentrated. The product was purified on silica gel (120 g, using 5% MeOH, 10% acetone in DCM as eluent) to provide the title compound (3.80 g, 100%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75 (br s, 1H), 0.84−1.22 (m, 7H), 1.21−1.35 (m, 2H), 1.39 (br s, 2H), 1.50 (br s, 1H), 1.91 (br s, 1H), 2.38 (br s, 2H), 3.33 (br s, 6H), 3.50 (br s, 4H), 5.07 (s, 2H), 7.23−7.44 (m, 5H). MS (ES+) m/z $[M + H]^+$ = 400.4. $[\alpha]_D^{20}$ = −12.9 (c 2.052, MeOH).

Methyl 2-(Bi(cyclohexan)-4-ylidene)acetate (16). To pentane-washed NaH 60% in mineral oil (0.31 g, 7.77 mmol) were added THF (25 mL) and methyl 2-(dimethoxyphosphoryl)acetate (1.20 mL, 8.32 mmol) dropwise over 20 min, resulting in a very thick suspension. After the reaction ceased bubbling, bi(cyclohexan)-4-one **15** (1.00 g, 5.55 mmol) (5 mL THF solution) was added. After 1 h, the reaction was filtered and washed with cold EtOAc. The filtrate was removed of solvent and put under high vacuum. The material was chromatographed (80 g SiO_2, hexanes to 75% CH_2Cl_2/hexanes to give the title compound as a clear oil (0.950 g, 72%). The material was used without further purification.

6-Methyl 6-Cyclohexylspiro[2.5]octane-1-carboxylate (17). To unwashed NaH 60% in mineral oil (0.040 g, 1.02 mmol) was added DMF (2 mL) followed by portionwise addition of solid trimethylsulfoxonium iodide (0.233 g, 1.06 mmol). The reaction was stirred for 1.5 h. To this was added methyl 2-(bi(cyclohexan)-4-ylidene)acetate **16** (0.10 mg, 0.42 mmol) in 1 mL of DMF. A fresh suspension of NaH/DMF (0.04 g, 1.02 mmol) and trimethylsulfoxo-

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

nium iodide (0.23 g, 1.06 mmol) was prepared and stirred for 10 min, which was then added to the first reaction. The reaction was stirred at room temperature for 48 h, and the solvent was removed. The white solids were triturated with CH_2Cl_2, and salts were filtered off. The crude material was chromatographed (SiO_2 20 g, gradient from pure hexanes to 1:1 hexanes/CH_2Cl_2) to give the title compound (0.52 g 49%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80−0.95 (m, 4H), 1.05−1.27 (m, 7H), 1.40−1.43 (m, 1H), 1.71−1.79 (m, 11H), 3.83 (s, 3H). ^{13}C MNR δ 20.57, 25.63, 26.86, 28.53, 28.94, 30.34,31.02, 37.29, 42.88, 42.97, 51.47, 173.49.

***tert*-Butyl 1,1-Dichloro-2-oxo-7-azaspiro[3.5]nonane-7-carboxylate (19).** To a suspension of zinc−copper couple (35.4 g) and *tert*-butyl 4-methylenepiperidine-1-carboxylate **18** (8.0 g, 40 mmol) in ether (150 mL) was added a solution of trichloroacetyl chloride (24.32 g, 130 mmol) in 1,2-dimetoxy ethane (40 mL) dropwise at room temperature under a nitrogen atmosphere. After stirring the mixture at room temperature for 6 h, the reaction mixture was poured into a saturated solution of $NaHCO_3$ (150 mL) at 0 °C and filtered. The filtrate was extracted with EtOAc (300 mL × 2), and the combined organic layer was washed with brine (150 mL), dried, and evaporated. The crude material was purified by column chromatography over silica gel using 20% of EtOAc in petroleum ether as an eluent to yield the title dichloro ketone (5.3 g, 40%) as a pale brown oil, which was used in the next step without further characterization.

***tert*-Butyl 2-Oxo-7-azaspiro[3.5]nonane-7-carboxylate (20).** To a solution of *tert*-butyl 1,1-dichloro-2-oxo-7-azaspiro[3.5]nonane-7-carboxylate (5.3 g, mmol) in saturated NH_4Cl and MeOH (80 mL) was added zinc (6.0 g) portionwise at room temperature. The reaction mixture was stirred at rt for 10 h and filtered. The filtrate was concentrated under vacuum, and the crude material was purified by column chromatography over silica gel using 20% of EtOAc in petroleum ether as an eluent to yield the title compound (4.0 g, 97%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.70 (t, J = 5.6 Hz, 4H), 2.81 (s, 4H), 3.41 (t, J = 5.6 Hz, 4H).

***tert*-Butyl 2-(Methylsulfonyloxy)-7-azaspiro[3.5]nonane-7-carboxylate (21).** To a solution of *tert*-butyl 2-oxo-7-azaspiro[3.5]-nonane-7-carboxylate **20** (3.00 g, 12.5 mmol) in MeOH (30 mL) was added $NaBH_4$ (0.57 g 15.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (80 mL), washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated to give the intermediate alcohol (3.0 g, 99%) as a white solid, which was used without further purification. To a solution of *tert*-butyl 2-hydroxy-7-azaspiro[3.5]nonane-7-carboxylate (3.50 g, 14.5 mmol) and Et_3N (4 mL, 29 mmol) in CH_2Cl_2 (50 mL) was added methane sulphonyl chloride (1.35 mL, 17.4 mmol) at 0−5 °C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured onto water (50 mL) and extracted with CH_2Cl_2. The combined organic layer was washed with water (20 mL) and brine (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give the title compound (4.5 g, 97%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.52−1.63 (m, 4H), 2.04−2.12 (m, 2H), 2.40−2.45 (m, 2H), 3.00 (s, 3H), 3.25−3.37 (m, 4H), 5.00−5.08 (m, 1H).

***tert*-Butyl 2-Cyano-7-azaspiro[3.5]nonane-7-carboxylate (22).** A mixture of *tert*-butyl 2-(methylsulfonyloxy)-7-azaspiro[3.5]-nonane-7-carboxylate (4.50 g, 14 mmol), sodium cyanide (1.35 g, 28 mmol), TBAB (20 mg), and DMF (40 mL) was heated at 120 °C and stirred for 16 h. The reaction mixture was poured into water (200 mL) and extracted with EtOAc (100 mL × 2). The combined organic layer was washed with water and brine, dried, and filtered, and the solvent was removed under reduced pressure. The crude material was purified by column chromatography over silica gel using 20% of EtOAc in petroleum ether as an eluent to yield the title compound (2.0 g, 57%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.55 (m, 2H), 1.64 (m, 2H), 2.19−2.25 (m, 4H), 3.1 (m, 1H), 3.30−3.32 (m, 4H).

7-*tert*-Butyl 2-Ethyl 7-azaspiro[3.5]nonane-2,7-dicarboxylate (23). To a solution of *tert*-butyl 2-cyano-7-azaspiro[3.5]nonane-7-carboxylate **22** (1.4 g, mmol) in ethanol (25 mL) was slowly added sulfuric acid (10 mL), and the mixture was refluxed for 16 h. The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was added to ice water (20 mL) and treated with sat. $NaHCO_3$ until basic, and a solution of Boc anhydride (1.54 g, 7.1 mmol) in THF (25 mL) was added at 5−10 °C. The mixture was allowed to come to room temperature and stirred for 16 h. The reaction mixture was extracted with EtOAc (50 mL × 2). The combined organic layer was washed with water and brine, dried, and concentrated. The crude material was purified by column chromatography over silica gel using 0−10% of EtOAc in petroleum ether as an eluent to afford the title compound (0.7 g, 41%) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, 3H, J = 7.12 Hz), 1.45 (s, 9H), 1.51−1.62 (m, 4H), 2.06 (d, J = 8.76 Hz, 4H), 3.08 (m, 1H), 3.27 (m, 2H), 3.35 (m, 2H), 4.14 (q, J = 7.12 Hz, 1H).

Benzyl 1,1-Dichloro-2-oxo-7-azaspiro[3.5]nonane-7-carboxylate (24). In a 500 mL round-bottomed flask were added benzyl 4-methylenepiperidine-1-carboxylate (11.0 g, 47.5 mmol) and zinc−copper couple (41.9 g, 642.0 mmol) combined in ether (150 mL) to give a gray suspension. The reaction suspension was cooled to 0 °C. In a separate flask were combined trichloroacetyl chloride (28.7 mL, 256.8 mmol) and DME (85 mL), which was then transferred to a dropping funnel. This solution was added dropwise to the round-bottomed flask reaction over 30 min. Moderate gas evolution with an exotherm was noted during the acid chloride addition. The reaction was stirred at 0 °C for 1 h and allowed to warm to room temperature by removing the ice bath. The reaction was stirred at room temperature for an additional 18 h. The reaction was quenched by slowly pouring the reaction mixture into a saturated $NaHCO_3$ solution (500 mL), which was kept at 0 °C. During the addition, vigorous gas evolution occurred. The quenched reaction was filtered through a bed of Celite, and all solids were removed. The filtrate was transferred to a separatory funnel and partitioned with EtOAc (1 L). The aqueous layer was back-extracted with EtOAc (2 × 250 mL). The combined organic layers were washed with brine (2 × 500 mL) and dried over $MgSO_4$. The organic layer was concentrated, and the resultant material was purified by SiO_2 chromatography (SiO_2, 330 g column, 100% hexane to 50% EtOAc/hexane over 30 min). The fractions were collected and evaporated to give the title compound (6.50 g, 40%). MS (ES+) (m/z) = 342, (m/z + 2) = 344. The material was used as is without further characterization.

Benzyl 2-Oxo-7-azaspiro[3.5]nonane-7-carboxylate (25). In a 1 L round-bottomed flask were added benzyl 1,1-dichloro-2-oxo-7-azaspiro[3.5]nonane-7-carboxylate (6.45 g, 18.8 mmol) and MeOH (50 mL) to give an orange solution. To this was added a solution of saturated NH_4Cl/MeOH (125 mL) followed by zinc dust (12.94 g, 197.9 mmol) in 6 equal portions over 15 min. The reaction was stirred at room temperature overnight. The solvent was removed by rotary evaporation, leaving behind a thick, yellow syrup. To this was added CH_2Cl_2 (100 mL), and the solids were filtered off followed by further concentration of the organics to give a yellow syrup. The residue was purified via SiO_2 chromatography (120 g column, 100% hexane to 50% EtOAc/hexane over 30 min) to give 3.85 g (74%) of the title compound as a pale oil. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 1.71 (m, 4H), 2.90 (s, 4H), 3.48 (m, 4H), 5.13 (s, 2H), 7.28−7.36 (m, 5H).

Benzyl 2-Formyl-7-azaspiro[3.5]nonane-7-carboxylate (26). Potassium *tert*-butoxide (1 M in THF, 77 mL, 0.77 mol) was loaded into an oven-dried flask under a nitrogen atmosphere. To this were added *t*-BuOH (7.38 mL, 77.16 mmol) and THF (70 mL). The mixture was cooled to −78 °C, and methoxymethyltriphenylphosphonium chloride (26.50 g, 77.16 mmol) was added portionwise. The reaction mixture was allowed to warm to room temperature, stirred for 1 h, and cooled back to −78 °C. A solution of benzyl 2-oxo-7-azaspiro[3.5]nonane-7-carboxylate **25** (7.03 g, 25.72 mmol) in THF (70 mL) was added dropwise to the reaction mixture over a period of 30 min. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The reaction was quenched with water (150 mL), and the product was extracted using EtOAc. The combined organic phases were washed with brine and dried over anhydrous $MgSO_4$, and the solvent was concentrated. The product was purified

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

on SiO_2 (330 g, 20% EtOAc in heptane as eluent) to provide the title compound (3.53 g, 47.8%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 1.48 (br s, 2H), 1.62 (br s, 2H), 1.91−2.16 (m, 4H), 3.16 (quin, *J* = 8.6 Hz, 1H), 3.31−3.41 (m, 2H), 3.41−3.50 (m, 2H), 5.12 (s, 2H), 7.28−7.44 (m, 5H), 9.76 (d, *J* = 1.56 Hz, 1H). MS (ES+) *m*/*z* $[M + H]^+$ = 288.1.

7-(Benzyloxycarbonyl)-7-azaspiro[3.5]nonane-2-carboxylic Acid (27). A solution of 6% aqueous sodium hypochlorite (84.0 g, 1.12 mol) was added to a solution of benzyl 2-formyl-7-azaspiro[3.5]-nonane-7-carboxylate (3.53 g, 12.28 mmol), $NaHCO_3$ (saturated) (8 mL), sodium bromide (253 mg, 2.46 mmol), and TEMPO (6 mg, 0.04 mmol, in CH_2Cl_2 6 mL solution) in water (30 mL) over a period of 2 h. The reaction mixture was stirred for 2 h, and the pH was adjusted to ∼1 by addition of concentrated hydrochloric acid. The product was extracted using Et_2O (3 × 70 mL). The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4. The solvent was concentrated, and the product was purified on SiO_2 (120 g, eluting with a 3:2 mixture of heptane/EtOAc) to afford 7-(benzyloxycarbonyl)-7-azaspiro[3.5]nonane-2-carboxylic acid (2.69 g, 72.2%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 (d, *J* = 16.8 Hz, 4H), 2.10 (d, *J* = 8.9 Hz, 4H), 3.13 (quin, *J* = 8.8 Hz, 1H), 3.33−3.41 (m, 2H), 3.41−3.48 (m, 2H), 5.12 (s, 2H), 7.28−7.41 (m, 5 H). MS (ES+) *m*/*z* $[M + H]^+$ = 304.2.

Ethyl 7-(Tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonane-2-carboxylate (28). To 7-*tert*-butyl 2-ethyl 7-azaspiro[3.5]nonane-2,7-dicarboxylate **23** (0.815 g, 2.74 mmol) in CH_2Cl_2 (2.7 mL) was added TFA (2.7 mL) to give an amber solution. The reaction was stirred at room temperature for 3 h, and the solvent was removed. The residue was triturated with ether (1 × 25 mL) and placed on the vacuum manifold for 1 h to give roughly 0.95 g of crude TFA salt. The salt was redissolved in CH_2Cl_2 (20 mL), and to this was added Si-Amine resin (∼2g, Silicycle, loading of 1.67 mmol/g). The heterogeneous mixture was stirred for 30 min at room temperature. The silica gel was filtered and washed with CH_2Cl_2, and the solvent was removed. The compound was placed under high vacuum for 8 h to give the intermediate piperidine as a waxy solid (0.541 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, *J* = 7.2 Hz, 3H), 1.69 (m, 4H), 2.06 (d, *J* = 8.7 Hz, 4H), 2.41 (br s, N-H/TFA-H), 2.82 (app t, *J* = 5.7 Hz, 2H), 2.88 (app t, *J* = 5.4 Hz, 2H), 3.08 (quintet, *J* = 8.7 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 2H). MS (ES+) *m*/*z* $[M]^+$ = 198.0. To ethyl 7-azaspiro[3.5]nonane-2-carboxylate (0.47 g, 2.38 mmol) were added MeOH (20 mL), dihydro-2*H*-pyran-4(3*H*)-one (0.80 mL, 9.53 mmol), and Pd/C (0.05 g, 0.05 mmol), and a balloon was attached to the reaction flask filled with H_2. The reaction was stirred at room temperature overnight. The reaction was filtered, and the solvent was removed in vacuo. The material was purified using SiO_2 chromatography (80 g, solvent gradient of 0−10% MeOH in CH_2Cl_2) to give the title compound (0.39 g, 1.40 mmol, 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (t, *J* = 7 Hz, 3H), 1.56−1.86 (br m, 10H), 2.01 (d, *J* = 8.5 Hz, 4H), 3.03 (m, 1H), (br m, 4H), 3.36 (app dt, *J* = 12.0, 1.5 Hz, 2H), 4.01 (dd, *J* = 11.0, 4.0 Hz, 2H), 4.12 (q, *J* = 7.0 Hz, 2H). MS (ES +) *m*/*z* $[M]^+$ = 282.4.

(4-Cyclobutyl-1,4-diazepan-1-yl)(7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonan-2-yl)methanone (29a). The title compound was prepared as in example **29c** in 39% yield as a film. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.97−0.99 (m, 6H), 1.56−1.61 (m, 6H), 1.61−1.68 (m, 2H), 1.71−1.76 (m, 2H), 1.78−1.81 (m, 2H), 1.94−1.97 (m, 2H), 2.07−2.11 (m, 2H), 2.40−2.47 (m, 2H), 2.55 (s, 1H), 2.55−2.58 (m, 2H), 2.62−2.66 (m, 2H), 2.85−2.91 (quin, *J* = 7.0 Hz, 1H), 3.12−3.17 (m, 1H), 3.36−3.40 (m, 4H), 3.57−3.60 (m, 2H), 3.99 (d, 1H, *J* = 4.5 Hz), 4.00 (d, 1H, *J* = 4.5 Hz). HRMS (TOF) *m*/*z* calcd for $C_{23}H_{40}N_3O_2$ $(M + H)^+$, 390.3115; found, 390.3114.

(4-Cyclobutylpiperazin-1-yl)(7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonan-2-yl)methanone (29b). To 7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonane-2-carboxylic acid (as prepared in example **29c**) (0.128 g, 0.39 mmol) were added DMF (3 mL), HBTU (0.150 g, 0.390 mmol), and then DIPEA (0.345 mL, 1.97 mmol). The reaction was stirred for 5 min, and 1-cyclobutylpiperazine dihydrochloride (0.126 g, 0.59 mmol) was added. The reaction was stirred at room temperature for 2 h, and the solvent was removed. The solids were put under high vacuum for 12 h. The material was subjected to SiO_2 chromatography (80 g, using a gradient of 0−10% 7 N NH_3/MeOH in CH_2Cl_2). The impure fractions were collected and further purified with preparatory HPLC using procedure A to provide the title compound as a white solid (0.112 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.68−1.83 (m, 12H), 1.85−2.05 (m, 7H), 2.23−2.28 (m, 4H), 2.35−2.47 (m, 4H), 2.70 (ddd, *J* = 11.4, 7.8, 7.8 Hz, 1H), 3.13 (ddd, *J* = 13.2, 9, 9 Hz, 1H), 3.31−3.38 (m, 4H), 3.59−3.62 (m, 2H), 3.99 (dd, *J* = 10.9, 3.9 Hz, 2H). HRMS (TOF) *m*/*z* calcd for $C_{21}H_{37}N_3O_2$ $(M + H)^+$, 376.2986; found, 376.2986.

(4-Cyclohexylpiperazin-1-yl)(7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonan-2-yl)methanone (29c). To ethyl 7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonane-2-carboxylate **28** (0.395 g, 1.40 mmol) were added MeOH (10 mL) and NaOH (1 N, 1.68 mL, 1.68 mmol). The reaction was heated to reflux for 2 h. The reaction was quenched by adding HCl (1 N, 1.684 mL, 1.68 mmol), and then the solvent was removed. The tan foam was placed under high vacuum at 50 °C for 12 h to give the intermediate acid in quantitative yield, which was used as is in the next reaction without further purification. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.45−1.69 (m, 8H), 1.86−1.88 (m, 2H), 1.94−1.96 (m, 2H), 2.45−2.50 (m, 3H), 2.98−3.02 (m, 1H), 3.22−3.26 (m, 4H), 3.78−3.88 (m, 2H). MS (ES+) *m*/*z* $[M]^+$ = 254.3. To 7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonane-2-carboxylic acid (309 mg, 1.22 mmol) was added thionyl chloride (10 mL), and the cloudy reaction was stirred at room temperature. After 30 min, the solvent was removed under vacuum, and the resulting white solid was put under high vacuum for 4 h. A sample of this was taken (0.050 g) to make the title compound as follows. To 7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonane-2-carbonyl chloride (0.050 mg, 0.18 mmol) was added a solution of 1-cyclohexylpiperazine (46.4 mg, 0.28 mmol) and DIPEA (0.129 mL, 0.74 mmol) in CH_2Cl_2 (2M, 2.0 mL), and the reaction was stirred for 2 h. The solvent was removed, and the residue was dried under high vacuum at 50 °C overnight. The material was purified by reverse-phase HPLC using procedure A. The fractions were collected, the solvent was removed, and the product was dried under high vacuum at 50 °C overnight (0.074 g, 61%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.14−1.18 (m, 4H), 1.37−1.42 (m, 4H), 1.57−1.59 (m, 5H), 1.61−1.63 (m, 4H), 1.70−1.73 (m, 4H), 1.86 (app t, *J* = 10 Hz, 4H), 2.22−2.34 (m, 4H), 2.33−2.39 (m, 6H), 3.17−3.21 (m, 2H), 3.32−3.39 (m, 2H), 3.84 (d, *J* = 5.0 Hz, 1H), 3.85 (d, *J* = 5 Hz, 1H). HRMS (TOF) *m*/*z* calcd for $C_{24}H_{42}N_3O_2$ $(M + H)^+$, 404.3272; found, 404.3267.

(Dihydro-1*H*-pyrido[1,2-a]pyrazin-2(6*H*,7*H*,8*H*,9*H*,9a*H*)-yl)(7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonan-2-yl)-methanone (29d). The material was prepared in an analogous manner to **29c** using octahydro-1*H*-pyrido[1,2-*a*]pyrazine to give the title compound as a white solid (0.69 g, 32%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40−1.35 (m, 3H), 1.73−1.81 (m, 9H), 2.02−2.07 (m, 4H), 2.06−2.11 (m, 4H), 2.34−2.47 (m, 6H), 2.72−2.77 (m, 2H), 2.77−2.83 (m, 2H), 3.11 (t, *J* = 8.5 Hz, 1H), 3.20 (t, *J* = 12.0 Hz, 0.5 H), 3.42 (d, *J* = 13.5 Hz, 2H), 3.99 (d, *J* = 11.5 Hz, 0.5 H), 4.01 (dd, *J* = 11.0 Hz, 4.0 Hz, 2H), 4.30 (d, *J* = 13.0 Hz, 0.5 H), 4.51 (d, *J* = 13.5 Hz, 0.5 H). HRMS (TOF) *m*/*z* calcd for $C_{22}H_{37}N_3O_2$ $(M + H)^+$, 376.2958; found, 376.2954. The compound appears to exist as rotational isomers based on multiple signals that integrate to less than 1H. These signals coalesce upon heating in DMSO-d_6, which supports this hypothesis.

(4-Isopropyl-1,4-diazepan-1-yl)(7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonan-2-yl)methanone dihydrochloride (29e). The material was prepared in an analogous manner to **29c** using 1-isopropyl-1,4-diazepane to give the title compound in 34% yield. The material was converted to the di-HCl salt by addition of aq. 0.2 M HCl and lyophilization. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (d, *J* = 6.6 Hz, 6H), 1.61−1.80 (m, 2H), 1.78−2.14 (m, 9H), 2.69−3.20 (m, 3H), 3.20−3.44 (m, 8H), 3.43−3.81 (m, 10H), 3.95 (dd, *J* = 11.33, 3.91 Hz, 2H), 10.45 (br s, 1H). HRMS (TOF) *m*/*z* calcd for $C_{22}H_{40}N_3O_2$ $(M + H)^+$, 378.3115; found, 378.3108.

(4-Isopropylpiperazin-1-yl)(7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonan-2-yl)methanone (29f). To 7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonane-2-carboxylic acid (as prepared in

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

example **29c**) (0.309 g, 1.22 mmol) was added thionyl chloride (10 mL), and the cloudy reaction was stirred at room temperature. After 30 min, the solvent was removed, and the white solid was placed under high vacuum for 4 h. A sample of this was taken (0.050 g) to make the title compound as follows. To 7-(tetrahydro-2*H*-pyran-4-yl)-7-azaspiro[3.5]nonane-2-carbonyl chloride (0.050g, 0.18 mmol) was added a solution of 1-isopropylpiperazine (0.039 mL, 0.28 mmol) and DIPEA (0.129 mL, 0.74 mmol) in CH_2Cl_2 (2.0 mL). The reaction was stirred at room temperature for 2 h. The solvent was removed, and the gum was put under high vacuum at 50 °C overnight. The material was purified by reverse-phase HPLC using the exact methods as for previous compounds. The resulting gum was placed under high vacuum at 50 °C overnight to give the title compound as a white solid (0.043 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (d, J = 6.5 Hz, 6H), 1.58 (m, 4H), 1.65−1.67 (m, 2H), 1.71−1.74 (m, 2H), 1.96−1.98 (m, 2H), 2.08 (m, 2H), 2.39- 2.47 (br m, 9H), 3.11 (quintet, J = 9.0 Hz, 1H), 3.33−3.38 (m, 4H), 3.59−3.61 (m, 2H), 3.99 (d, J = 4.0 Hz, 1H), 4.01 (d, J = 4.0 Hz, 1H). HRMS (TOF) m/z calcd for $C_{21}H_{37}N_3O_2$ $(M + H)^+$, 364.2958; found, 364.2957.

(4-Cyclobutylpiperazin-1-yl)(7-(methylsulfonyl)-7-azaspiro-[3.5]nonan-2-yl)methanone (29g). Methanesulfonyl chloride (0.032 mL, 0.41 mmol) was added to a solution of (4-cyclobutylpiperazin-1-yl)(7-azaspiro[3.5]nonan-2-yl)methanone (0.100 g, 0.34 mmol) and triethylamine (52.1 mg, 0.51 mmol) in dichloromethane (10 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The solvent was concentrated. The product was purified on preparative HPLC using procedure B to provide the title compound (49.6 mg, 39.1%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59−1.72 (m, 3H), 1.72−1.80 (m, 3H), 1.79−1.94 (m, J = 10.1, 9.7, 9.5, 9.5 Hz, 2H), 1.95−2.10 (m, 4H), 2.09−2.20 (m, 2H), 2.22−2.34 (m, 4H), 2.66−2.74 (m, 1H), 2.76 (s, 3H), 3.05−3.15 (m, 2H), 3.14−3.26 (m, 3H), 3.30−3.38 (m, 2H), 3.62 (t, J = 4.8 Hz, 2H). HRMS (TOF) m/z calcd for $C_{18}H_{31}N_3O_3S$ $(M + H)^+$, 370.2158; found, 370.2160.

(4-Cyclobutylpiperazin-1-yl)(7-phenyl-7-azaspiro[3.5]-nonan-2-yl)methanone (29h). Cesium carbonate (0.123 g, 0.38 mmol) was added to a solution of (4-cyclobutylpiperazin-1-yl)(7-azaspiro[3.5]nonan-2-yl)methanone **31g** (0.100 g, 0.340 mmol), $Pd(OAc)_2$ (0.007 g, 0.03 mmol), BINAP (0.042 g, 0.07 mmol), and bromobenzene (0.056 g, 0.36 mmol) in toluene (5 mL). The reaction mixture was heated at 110 °C for 18 h. The reaction was cooled to room temperature and filtered over Celite. The solvent was concentrated, and the product was purified by preparative HPLC using procedure B to provide the title compound as white solid (59.0 mg, 46.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60−1.75 (m, 4H), 1.75−1.81 (m, 2H), 1.81−1.95 (m, 2H), 1.96−2.10 (m, 4H), 2.10−2.20 (m, 2H), 2.21−2.35 (m, 4H), 2.71 (dq, J = 8.0, 7.7 Hz, 1H), 3.00−3.11 (m, 2H), 3.11−3.26 (m, 3H), 3.31−3.42 (m, 2H), 3.56−3.70 (m, 2H), 6.82 (t, J = 7.2 Hz, 1H), 6.93 (d, J = 7.81 Hz, 2H), 7.18−7.29 (m, 2H). HRMS (TOF) m/z calcd for $C_{23}H_{33}N_3O$ $(M + H)^+$, 368.2696; found, 368.2698.

(7-Benzoyl-7-azaspiro[3.5]nonan-2-yl)(4-cyclobutylpiperazin-1-yl)methanone (29i). Benzoyl chloride (0.035 mL, 0.30 mmol) was added to a solution of (4-cyclobutylpiperazin-1-yl)(7-azaspiro[3.5]nonan-2-yl)methanone **31g** (80 mg, 0.27 mmol) and DIPEA (0.058 mL, 0.33 mmol) in dichloromethane (8 mL). The reaction mixture was stirred for 3 days, and the solvent was concentrated. The crude material was purified on preparative HPLC MS using procedure B to provide the title compound as white solid (36.5 mg, 33.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (br s, 1H), 1.62−1.68 (m, 2H), 1.68−1.80 (m, 3H), 1.80−1.95 (m, 2H), 1.96−2.11 (m, 4H), 2.16 (br s, 2H), 2.27 (q, J = 5.47 Hz, 4H), 2.71 (dq, J = 8.0, 7.7 Hz, 1H), 3.11−3.30 (m, 2H), 3.35 (br s, 3H), 3.55−3.67 (m, 3H), 3.72 (br s, 1H), 7.32−7.44 (m, 5H). HRMS (TOF) m/z calcd for $C_{24}H_{33}N_3O_2$ $(M + H)^+$, 396.2645; found, 396.2650.

(7-Benzoyl-7-azaspiro[3.5]nonan-2-yl)(4-cyclobutyl-1,4-diazepan-1-yl)methanone (29j). Benzoyl chloride (0.040 mL, 0.34 mmol) was added to a solution of (4-cyclobutyl-1,4-diazepan-1-yl)(7-azaspiro[3.5]nonan-2-yl)methanone **31g** (0.095 g, 0.31 mmol) and DIEA (0.081 mL, 0.47 mmol) in dichloromethane (10 mL). The reaction mixture was stirred overnight, and the solvent was concentrated. The crude material was purified on preparative HPLC using procedure B to provide the title compound as a white solid (0.082 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (br s, 1H), 1.56−1.72 (m, 4H), 1.72−1.95 (m, 5H), 1.96−2.11 (m, 4H), 2.17 (br s, 2H), 2.35−2.45 (m, 2H), 2.45−2.54 (m, 2H), 2.76−2.91 (m, 1H), 3.26 (br s, 2H), 3.34 (br s, 1H), 3.42 (t, J = 5.86 Hz, 2H), 3.55−3.68 (m, 3H), 3.72, (br s, 1H), 7.31−7.49 (m, 5H). HRMS (TOF) m/z calcd for $C_{25}H_{35}N_3O_2$ $(M + H)^+$, 410.2802; found, 410.2797.

Benzyl 2-(4-Cyclobutylpiperazine-1-carbonyl)-7-azaspiro[3.5]nonane-7-carboxylate (30g). HBTU (1.018 g, 2.68 mmol) and 1-cyclobutylpiperazine (342 mg, 2.44 mmol) were added to a solution of 7-(benzyloxycarbonyl)-7-azaspiro[3.5]nonane-2-carboxylic acid (0.74 g, 2.44 mmol) and DIEA (0.511 mL, 2.93 mmol) in DMF (60 mL). The reaction mixture was stirred for 3 h, and the solvent was concentrated. The product was purified on silica gel by using 3% MeOH and 5% acetone in CH_2Cl_2 with 0.1 N ammonia as the eluent (80 g column; 20 mL/min then 40 mL/min) to provide the title compound (0.81 g, 78%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43−1.57 (m, 3H), 1.56−1.67 (m, 2H), 1.67−1.82 (m, 2H), 1.82−1.98 (m, 3H), 1.98−2.08 (m, 3H), 2.08−2.18 (m, 2H), 2.26−2.35 (m, 3H), 2.69−2.80 (m, J = 8.20, 7.91, 7.76, 7.76 Hz, 1H), 3.18 (quin, J = 8.50 Hz, 1H), 3.33−3.42 (m, 4H), 3.41−3.50 (m, 2H), 3.58−3.69 (m, 2H), 5.12 (s, 2H), 7.29−7.41 (m, 5H). MS m/z (ES+) $[M + H]^+$ = 426.3.

Benzyl 2-(4-Cyclobutyl-1,4-diazepane-1-carbonyl)-7-azaspiro[3.5]nonane-7-carboxylate (30j). Oxalyl chloride (0.970 mL, 11.08 mmol) was slowly added to a solution of 7-(benzyloxycarbonyl)-7-azaspiro[3.5]nonane-2-carboxylic acid (1.12 g, 3.69 mmol) in dichloromethane (50 mL) at 0 °C under a nitrogen atmosphere. The solution was stirred for 4 h while gradually warming to 15 °C. The solvent was concentrated under vacuum, and the residue was recovered in CH_2Cl_2 (10 mL). The resulting solution was added dropwise over 15 min to a solution of 1-cyclobutyl-1,4-diazepane (0.62 g, 4.06 mmol) and DIEA (1.934 mL, 11.08 mmol) in CH_2Cl_2 (50 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 30 min and concentrated. The product was purified on SiO_2 (gradient of MeOH 3% in CH_2Cl_2 (500 mL) to MeOH 5% in CH_2Cl_2 (500 mL)). Impure fractions were resubjected to purification under the same conditions to give several batches of pure compound, which were combined to provide the title compound as a white solid (0.681 g, 42.0%). t_R = 1.80 min (5 min run). MS m/z (ES+) $[M + H]^+$ = 440.39. The material was used is as without further characterization.

(4-Cyclobutylpiperazin-1-yl)(7-azaspiro[3.5]nonan-2-yl)-methanone (31g). A mixture of benzyl 2-(4-cyclobutylpiperazine-1-carbonyl)-7-azaspiro[3.5]nonane-7-carboxylate (0.80 g, 1.88 mmol), Pd/C (0.01 g, 0.09 mmol), and ethanol (100 mL) was shaken in a Parr apparatus under a 50 psi atmosphere of hydrogen for 4 h. The mixture was filtered over a Celite bed, and the solvent was concentrated to provide (4-cyclobutylpiperazin-1-yl)(7-azaspiro[3.5]nonan-2-yl)-methanone (480 mg, 88%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49−1.62 (m, 2H), 1.62−1.78 (m, 3H), 1.79−1.95 (m, J = 9.4, 9.4, 9.2, 8.9 Hz, 2H), 1.95−2.16 (m, 5H), 2.27 (q, J = 5.1 Hz, 4H), 2.60 (br s, 3H), 2.65−2.74 (m, 1H), 2.74−2.81 (m, 2H), 2.81−2.93 (m, 2H), 3.16 (quin, J = 8.7 Hz, 1H), 3.28−3.40 (m, 2H), 3.62 (t, J = 4.9 Hz, 2H). MS m/z (ES+) $[M + H]^+$ = 292.2.

(4-Cyclobutyl-1,4-diazepan-1-yl)(7-azaspiro[3.5]nonan-2-yl)-methanone (31j). The title compound was prepared in an analogous manner to **31g** to yield a white solid (1.62 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49−1.58 (m, 2H), 1.58−1.74 (m, 3H), 1.74−1.93 (m, 4H), 1.93−2.17 (m, 8H), 2.36−2.45 (m, 2H), 2.48 (dt, J = 4.98, 2.15 Hz, 2H), 2.67−2.78 (m, 2H), 2.78−2.91 (m, 3H), 3.17 (quin, J = 8.79 Hz, 1H), 3.37−3.47 (m, 2H), 3.5 −3.67 (m, 2H).

***tert*-Butyl 2-(4-Isopropylpiperazine-1-carbonyl)-2,7-diazaspiro[3.5]nonane-7-carboxylate (33).** To a −78 °C cooled solution of 4-nitrophenyl carbonochloridate (0.384 g, 1.90 mmol) in THF (5 mL) was added DIPEA (1.00 mL, 5.71 mmol) followed by 1-isopropylpiperazine (0.28 mL, 1.90 mmol). This cloudy mixture was stirred 5 min, and then *tert*-butyl 2,7-diazaspiro[3.5]nonane-7-carboxylate hydrochloride (0.50 g, 1.90 mmol) was added. The

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

reaction was warmed to room temperature and then refluxed for 12 h. The crude reaction was partitioned between EtOAc/$NaHCO_3$ (sat.). The organic layer was dried over Na_2SO_4, and the solvent was removed to give an amber gum. The material was chromatographed with SiO_2 chromatography (80 g, using a gradient of 0−10% MeOH to CH_2Cl_2) and dried to give a tan solid (0.525 g, 72%). ^{1}H NMR 500 MHz, $CDCl_3$) δ 1.03 (d, J = 6.5 Hz, 6H), 1.45 (s, 9H), 1.69 (app t, J = 5.5 Hz, 4H), 2.47 (br s, 4H), 2.65 (m, 1H), 3.35−3.33 (m, 8H), 3.70 (s, 4H). MS m/z (ES+) $[M + H]^+$ = 381.3.

(4-Isopropylpiperazin-1-yl)(7-(tetrahydro-2*H*-pyran-4-yl)-2,7-diazaspiro[3.5]nonan-2-yl)methanone (34). To *tert*-butyl 2-(4-isopropylpiperazine-1-carbonyl)-2,7-diazaspiro[3.5]nonane-7-carboxylate (0.51 g, 1.34 mmol) was added MeOH (20 mL) that had been saturated with HCl gas. The reaction was stirred 1 h, the solvent was removed, and the white solid was put under high vacuum for 12 h (0.52 g). MS m/z (ES+) $[M + H]^+$ = 281.2. The material contained ∼50% impurity that was not removed but was used as is in the next reaction without further purification. To impure (4-isopropylpiperazin-1-yl)(2,7-diazaspiro[3.5]nonan-2-yl)methanone dihydrochloride (0.52 g, 1.49 mmol) were added MeOH (10 mL), TEA (0.621 mL, 4.46 mmol), Pd/C (63.3 mg, 0.06 mmol), and dihydro-2*H*-pyran-4(3*H*)-one (0.55 mL, 5.94 mmol). The reaction was fitted with a balloon filled with H_2 and stirred for 24 h. The catalyst was filtered off, the solvent was removed under reduced pressure, and the resulting solids were collected. The material was purified with preparatory HPLC procedure A to provide the title compound as a white solid (0.155 g, 28%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.06 (s, 6H), 1.76−1.80 (m, 2H), 1.79−1.87 (m, 2H), 2.07−2.09 (2H), 2.34−2.35 (m, 2H), 2.47 (app t, J = 5 Hz, 4H), 2.64 (quintet, J = 6.5 Hz, 1H), 3.48−3.52 (m, 8H), 3.58−3.61 (m, 6H), 3.91−3.94 (m, 1H), 4.08 (dd, J = 11.7, 4.5 Hz, 2H). HRMS (TOF) m/z calcd for $C_{20}H_{36}N_4O_2$ $(M + H)^+$, 365.2911; found, 365.2913.

***tert*-Butyl 2-(4-Cyclobutyl-1,4-diazepane-1-carbonyl)-2,7-diazaspiro[4.5]decane-7-carboxylate (36a).** *tert*-Butyl 2,7-diazaspiro[4.5]decane-7-carboxylate hydrochloride **35** (2.30 g, 8.32 mmol) was dissolved in dry THF (40 mL), and to this was added freshly recrystallized carbonyl diimidazole (1.48 g, 9.15 mmol). The reaction mixture was heated at reflux for 24 h, and then THF was removed in vacuo. The residue was dissolved in EtOAc (75 mL), washed with 5% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford the title imidazole intermediate (3.13 g, 113%) as a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.49−1.98 (m, 6H), 3.12−3.83 (m, 8H), 7.08 (s, 1H), 7.33 (s, 1H), 8.00 (s, 1H). This intermediate (2.78 g, 8.31 mmol) was dissolved in acetonitrile (20 mL), MeI (4.72 g, 33.25 mmol) was added, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated to dryness to yield the intermediate imidazolium salt as a yellow solid (4.59 g, 158%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.45 (s, 9H), 1.51−2.03 (m, 6H), 3.34−3.91 (m, 8H), 7.72 (s, 1H), 8.04 (s, 1H), 9.52 (s, 1H). In a round-bottomed 50 mL flask were added the imidazolium salt (738 mg, 2.11 mmol), CH_2Cl_2 (10 mL), and Et_3N (0.24 mL, 1.72 mmol). Another flask was charged with 1-cyclobutyl-1,4-diazepane dihydrochloride (400 mg, 1.76 mmol), CH_2Cl_2 (10 mL), and Et_3N (0.50 mL, 3.56 mmol). Both mixtures were stirred 15 min, and the amine solution was transferred via syringe into the first flask. The reaction mixture was stirred 24 h at room temperature and then washed with $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified via preparatory HPLC using procedure C to produce the title compound (392 mg, 40%) as a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (s, 9H), 1.33−1.91 (m, 12H), 1.91−2.05 (m, 2H), 2.27−2.59 (m, 4H), 2.73−2.88 (m, 1H), 2.98−3.54 (m, 12H).

***tert*-Butyl 2-(4-Isopropylpiperazine-1-carbonyl)-2,7-diazaspiro[4.5]decane-7-carboxylate (36b).** The title compound was prepared in an analogous fashion to **36a** beginning with 1-isopropylpiperazine dihydrochloride to produce the title compound as a yellowish oil (392 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (d, J = 6.5 Hz, 6H), 1.33 (s, 9H), 1.33−1.54 (m, 5H), 1.57−1.73 (m, 1H), 2.30−2.45 (m, 4H), 2.51−2.64 (m, 1H), 2.94−3.47 (m, 12H).

(4-Cyclobutyl-1,4-diazepan-1-yl)(7-(tetrahydro-2*H*-pyran-4-yl)-2,7-diazaspiro[4.5]decan-2-yl)methanone (37a). *tert*-Butyl 2-(4-cyclobutyl-1,4-diazepane-1-carbonyl)-2,7-diazaspiro[4.5]decane-7-carboxylate **36a** (400 mg, 0.95 mmol) was dissolved in 6 N HCl *i*PrOH (5 mL) and stirred overnight at room temperature. The reaction mixture was concentrated to dryness, CH_2Cl_2 (10 mL) was added followed by Et_3N (0.29 mL, 2.08 mmol), and the reaction mixture was stirred 10 min. Dihydro-2*H*-pyran-4(3*H*)-one (156 mg, 1.56 mmol) and AcOH (56 μL, 0.95 mmol) were added. After 15 min of stirring, $NaBH(OAc)_3$ (331 mg, 1.56 mmol) was added, and the reaction mixture was left stirring overnight at room temperature. The reaction mixture was washed with 5% $NaHCO_3$, extracted with CH_2Cl_2 (3×), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified via preparatory HPLC using procedure C to produce the title compound (150 mg, 36%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18−2.01 (m, 19H), 2.18 (dd, J = 28.1, 10.9 Hz, 2H), 2.28−2.53 (m, 6H), 2.79 (m, 1H), 2.99 (d, J = 10.3 Hz, 1H), 3.16−3.40 (m, 7H), 3.66 (s, 2H), 3.82−3.93 (m, 2H). HRMS (TOF) m/z calcd for $C_{23}H_{40}N_4O_2$ $(M + H)^+$, 405.3229; found, 405.3224.

(4-Isopropylpiperazin-1-yl)(7-(tetrahydro-2*H*-pyran-4-yl)-2,7-diazaspiro[4.5]decan-2-yl)methanone (37b). Material was prepared in an analogous manner to **37a** to provide the title compound (167 mg, 44%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (d, J = 6.5 Hz, 6H), 1.29−1.69 (m, 10H), 1.77−1.88 (m, 1H), 2.28 (q, J = 11.0 Hz, 2H), 2.38−2.58 (m, 6H), 2.61−2.74 (m, 1H), 3.12 (d, J = 10.4 Hz, 1H), 3.22 −3.52 (m, 9H), 3.98 (d, J = 9.4 Hz, 2H). HRMS (TOF) m/z calcd for $C_{21}H_{38}N_4O_2$ $(M + H)^+$, 379.3073; found, 379.3080.

(7-Benzoyl-2,7-diazaspiro[4.5]decan-2-yl)(4-cyclobutyl-1,4-diazepan-1-yl)methanone (37c). (4-Cyclobutyl-1,4-diazepan-1-yl)-(2,7-diazaspiro[4.5]decan-2-yl)methanone **36a** (200 mg, 0.51 mmol) was dissolved in CH_2Cl_2 (3 mL). Triethylamine (0.21 mL, 1.53 mmol) was added, and the solution was stirred at room temperature for 10 min. Benzoyl chloride (0.07 mL, 0.61 mmol) was then added, and the mixture was stirred at room temperature for 4 h. The solution was diluted with CH_2Cl_2 (10 mL) and washed with 5% aq. $NaHCO_3$ (1 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated. The product was purified through flash-chromatography (SiO_2, gradient of CH_2Cl_2/MeOH 100:0 to 80:20) and then on reverse phase (flow: 30 mL/min, H_2O/CH_3CN 100:0 to 5:95) to provide the title compound (103 mg, 48%) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44−2.16 (m, 13H), 2.21−2.65 (m, 6H), 2.75−2.92 (m, 1H), 2.99−3.71 (m, 10H), 3.72−3.94 (m, 1H), 7.30−7.45 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.4, 22.4, 23.6, 27.9, 33.9, 42.1, 46.6, 47.6, 47.8, 49.0, 51.4, 52.2, 54.2, 56.4, 56.7, 59.7, 126.8, 128.4, 129.4, 136.0, 163.0, 170.7. HRMS (TOF) m/z calcd for $C_{25}H_{36}N_4O_2$ $(M + H)^+$, 425.2911; found, 425.2909.

(4-Cyclobutyl-1,4-diazepan-1-yl)(7-(phenylsulfonyl)-2,7-diazaspiro[4.5]decan-2-yl)methanone (37d). (4-Cyclobutyl-1,4-diazepan-1-yl)(2,7-diazaspiro[4.5]decan-2-yl)methanone (200 mg, 0.51 mmol) was dissolved in CH_2Cl_2 (3 mL). Triethylamine (0.21 mL, 1.53 mmol) was added, and the solution was stirred at room temperature for 10 min. Benzenesulfonyl chloride (0.078 mL, 0.61 mmol) was then added, and the mixture was stirred at room temperature for 4 h. The solution was then was diluted with CH_2Cl_2 (10 mL) and washed with 5% aq. $NaHCO_3$ (1 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated. The product was purified through flash-chromatography (SiO_2, gradient of CH_2Cl_2/MeOH 100:0 to 80:20) and then on reverse phase (flow: 30 mL/min, H_2O/CH_3CN 100:0 to 5:95) to provide the title compound (117 mg, 50%) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38−1.44 (m, 2H), 1.52−1.73 (m, 5H), 1.77−1.94 (m, 4H), 1.98−2.07 (m, 2H), 2.17−2.28 (m, 1H), 2.35−2.48 (m, 2H), 2.50−2.57 (m, 2H), 2.66 (d, J = 11.4 Hz, 1H), 2.79−2.86 (m, 2H), 2.95 (d, J = 11.5 Hz, 1H), 3.05−3.10 (m, 1H), 3.18 (q, J = 10.9 Hz, 2H), 3.35−3.51 (m, 6H), 7.49−7.61 (m, 3H), 7.73 (d, J = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 22.6, 27.9, 28.1, 33,0, 33.8, 41.5, 46.6, 46.8, 47.6, 48.0, 51.5, 52.1, 53.1, 57.1, 59.7, 127.4, 129.0, 132.7, 136.3, 163.0. MS m/z (ES+) $[M + H]^+$ = 461.25.

dx.doi.org/10.1021/jm4014828 | *J. Med. Chem.* XXXX, XXX, XXX−XXX

HRMS (TOF) m/z calcd for $C_{24}H_{36}N_4SO_3$ $(M + H)^+$, 461.2580; found, 461.2576.

***tert*-Butyl 2-(4-Isopropylpiperazine-1-carbonyl)-2,8-diazaspiro[4.5]decane-8-carboxylate (40).** To a −78 °C cooled solution of 4-nitrophenyl carbonochloridate (0.36 g, 1.81 mmol) in THF (10 mL) was added DIPEA (0.95 mL, 5.42 mmol) and then 1-isopropylpiperazine (0.26 mL, 1.81 mmol). After stirring the mixture for 5 min, *tert*-butyl 2,8-diazaspiro[4.5]decane-8-carboxylate hydrochloride (0.500 g, 1.81 mmol) was added, and the reaction was warmed to room temperature. The reaction was refluxed overnight for 12 h. The material was chromatographed with SiO_2 chromatography (80 g, using a gradient of 0−10% MeOH to CH_2Cl_2) and dried to give the title compound as white solid (0.20 g, 28%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.05 (d, J = 6.5 Hz, 6H), 1.45 (s, 9H), 1.49−1.52 (m, 4H), 1.71 (t, 2H, J = 7 Hz, 2H), 2.48−2.50 (m, 4H), 2.67−2.69 (m, 1H), 3.22 (s, 2H), 3.31−3.36 (m, 6H), 3.46−3.42 (m, 4H). MS m/z (ES+) $[M + H]^+$ = 395.3.

***tert*-Butyl 2-(4-Cyclobutyl-1,4-diazepane-1-carbonyl)-2,8-diazaspiro[4.5]decane-8-carboxylate (40b).** *tert*-Butyl 2,8-diazaspiro[4.5]decane-8-carboxylate hydrochloride **39** (1.0 g, 3.61 mmol) was dissolved in dry THF (20 mL), and then freshly recrystallized carbonyl diimidazole (644 mg, 3.97 mmol) was added. The reaction mixture was heated at reflux for 24 h, and THF was removed in vacuo. The residue was dissolved in EtOAc (50 mL), washed with $NaHCO_3$ (5%) and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield the intermediate imidazole (1.45 g, 120%) as a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.50−1.65 (m, 4H), 1.89 (t, J = 7.1 Hz, 2 H), 3.28−3.57 (m, 6H), 3.73 (t, J = 7.1 Hz, 2H), 7.09 (s, 1H), 7.34 (s, 1H), 8.01 (s, 1H). This material (1.13 g, 2.71 mmol) was dissolved in acetonitrile (8 mL), MeI (1.54 g, 10.8 mmol) was added, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated to dryness, and to this were added CH_2Cl_2 (8 mL), Et_3N (1.13 mL, 8.13 mmol), and 1-cyclobutyl-1,4-diazepane (616 mg, 2.71 mmol). The reaction mixture was stirred 24 h at room temperature, washed with $NaHCO_3$ (sat.) brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified via preparatory HPLC using procedure C and then reverse-phase preparatory HPLC (H_2O/ACN, Flow: 30 mL/min, 12 g column, 100:0 to 0:100 over 30 min) to produce the title compound (742 mg, 65%) as a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37−1.56 (m, 13H), 1.56−1.76 (m, 6H), 1.76−1.95 (m, 4H), 1.95−2.09 (m, 2H), 2.37−2.57 (m, 4H), 2.77−2.91 (m, 1H), 3.21 (s, 2H), 3.24−3.37 (m, 2H), 3.37−3.54 (m, 6H).

***tert*-Butyl 2-(4-Cyclobutylpiperazine-1-carbonyl)-2,8-diazaspiro[4.5]decane-8-carboxylate (40e).** Crude *tert*-butyl 2-(1*H*-imidazole-1-carbonyl)-2,8-diazaspiro[4.5]decane-8-carboxylate (1.45 g, 3.61 mmol) was dissolved in acetonitrile (8 mL), MeI (2.05 g, 14.4 mmol) was added, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated to dryness, and to this were added CH_2Cl_2 (8 mL), Et_3N (1.51 mL, 10.83 mmol), and 1-cyclobutylpiperazine (808 mg, 3.79 mmol). The reaction mixture was stirred 24 h at room temperature and then washed with $NaHCO_3$ and NaCl (sat.), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified via preparatory HPLC using procedure C and then reverse-phase preparatory HPLC (H_2O/ACN, flow: 30 mL/min, 12 g column, 100:0 to 0:100 over 30 min) to produce the title compound (980 mg, 67%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37−1.54 (m, 13H), 1.60−1.78 (m, 4H), 1.78−1.95 (m, 2H), 1.95−2.09 (m, 2H), 2.24−2.36 (m, 4H), 2.64−2.78 (m, 1H), 3.22 (s, 2H), 3.25−3.39 (m, 6H), 3.39−3.52 (m, 4H).

(4-Isopropylpiperazin-1-yl)(8-(tetrahydro-2*H*-pyran-4-yl)-2,8-diazaspiro[4.5]decan-2-yl)methanone (41a). To *tert*-butyl 2-(4-isopropylpiperazine-1-carbonyl)-2,8-diazaspiro[4.5]decane-8-carboxylate (0.185 g, 0.47 mmol) was added MeOH (20 mL) that had been saturated with HCl gas. The reaction was stirred 1 h, the solvent was removed, and the white solid was put under high vacuum overnight to give the intermediate amine (0.120 g, 75%). ^{1}H NMR (500 MHz, DMSO-d_6, TFA shake) δ 1.24−1.25 (m, 6H), 1.61−1.67 (m, 4H), 1.75−1.77 (m, 2H), 2.98 (app t, J = 11.5 Hz, 2H), 2.98−3.00 (m, 4H), 3.15−3.18 (m, 2H), 3.22 (s, 2H), 3.40−3.48 (m, 5H), 3.49−3.57 (m, 1H), 3.75 (app d, J = 14.0 Hz, 2H). MS m/z (ES+) $[M + H]^+$ = 295.3. To (4-isopropylpiperazin-1-yl)(2,8-diazaspiro[4.5]decan-2-yl)methanone dihydrochloride (0.20 g, 0.54 mmol) were added MeOH (5 mL), dihydro-2*H*-pyran-4(3*H*)-one (0.20 mL, 2.18 mmol), TEA (0.23 mL, 1.63 mmol), and Pd/C (23.18 mg, 0.02 mmol), and a balloon filled with H_2 gas was fitted to the reaction. After stirring for 24 h, the catalyst was filtered off, the solvent was removed under reduced pressure, and solids were put under high vacuum. The material was purified with preparatory HPLC using procedure A to provide the title compound as a white solid (0.205 g, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03 (d, J = 6.5 Hz, 6H), 1.57−1.61 (m, 7H), 1.72 (t, J = 7.0 Hz, 2H), 1.70−1.75 (m, 1H), 2.42−2.49 (m, 7H), 2.57−2.58 (m, 2H), 2.67 (quin., J = 6.5 Hz, 1H), 3.19 (s, 2H), 3.30−3.28 (m, 4H), 3.36 (dt, J = 12, 2, Hz, 2H), 3.42 (t, J = 7.0 Hz, 2H), 4.00−4.03 (m, 2H). HRMS (TOF) m/z calcd for $C_{21}H_{38}N_4O_2$ $(M + H)^+$, 379.3067; found, 379.3055.

(8-Benzoyl-2,8-diazaspiro[4.5]decan-2-yl)(4-cyclobutyl-1,4-diazepan-1-yl)methanone (41b). To *tert*-butyl 2-(4-cyclobutyl-1,4-diazepane-1-carbonyl)-2,8-diazaspiro[4.5]decane-8-carboxylate **40b** (742 mg, 1.76 mmol) was added 6 N HCl in *i*PrOH (5 mL). The reaction mixture was stirred 4 h, and then Et_2O (50 mL) was added. The salt was filtered under N_2 and dried in vacuo to make the intermediate amine (755 mg, 108%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 1.72−2.01 (m, 7H), 2.15−2.43 (m, 6H), 2.92−3.28 (m, 7H), 3.35−3.90 (m, 11H). (4-Cyclobutyl-1,4-diazepan-1-yl)(2,8-diazaspiro[4.5]decan-2-yl)methanone (200 mg, 0.51 mmol) and Et_3N (212 μL, 1.52 mmol) were mixed in a flask in CH_2Cl_2 (3 mL) and stirred 10 min. Benzoyl chloride (73 mg, 0.52 mmol) was added, and the reaction mixture was stirred for 2 h. The reaction mixture was diluted with CH_2Cl_2 and washed with 5% $NaHCO_3$ and brine, then dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified via preparatory HPLC using procedure C and then reverse-phase preparatory HPLC (H_2O/ACN, flow: 30 mL/min, 12 g column, 100:0 to 0:100 over 30 min) to produce the title compound (200 mg, 85%) as a clear oil after lyophilization (H_2O/ACN). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36−1.93 (m, 12H), 1.93−2.10 (m, 2H), 2.28−2.47 (m, 3H), 2.47−2.57 (m, 2H), 2.74−2.90 (m, 1H), 3.25 (s, 2H), 3.25−3.50 (m, 7H), 3.50−3.72 (m, 1H), 3.72−3.94 (m, 1H), 7.32−7.44 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 27.9, 28.2, 34.0, 35.0, 35.8, 39.7, 40.2, 45.4, 46.7, 47.6, 48.0, 51.4, 52.1, 57.8, 59.7, 126.8, 128.4, 129.5, 136.0, 163.1, 170.3. HRMS (TOF) m/z calcd for $C_{25}H_{36}N_4O_2$ $(M + H)^+$, 425.2911; found, 425.2910.

(4-Cyclobutyl-1,4-diazepan-1-yl)(8-(phenylsulfonyl)-2,8-diazaspiro[4.5]decan-2-yl)methanone (41c). (4-Cyclobutyl-1,4-diazepan-1-yl)(2,8-diazaspiro[4.5]decan-2-yl)methanone **40b** (200 mg, 0.510 mmol) and Et_3N (212 μL, 1.52 mmol) were mixed in a flask in CH_2Cl_2 (3 mL) and stirred 10 min. Benzenesulfonyl chloride (92 mg, 0.52 mmol) was added, and the reaction mixture was stirred 2 h. The reaction mixture was diluted with CH_2Cl_2, washed with 5% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified via preparatory HPLC using procedure C and then reverse-phase preparatory HPLC (H_2O/ACN, flow: 30 mL/min, 12 g column, 100:0 to 0:100 over 30 min) to produce the title compound (180 mg, 77%) as a white solid after lyophilization (H_2O/ACN). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51−1.72 (m, 8H), 1.72−1.89 (m, 4H), 1.96−2.08 (m, 2H), 2.34−2.43 (m, 2H), 2.46−2.53 (m, 2H), 2.72−2.86 (m, 3H), 3.07 (s, 2H), 3.18−3.29 (m, 2H), 3.29−3.44 (m, 6H), 7.50−7.65 (m, 3H), 7.72−7.77 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 28.0, 28.2, 33.7, 36.2, 39.1, 43.7, 46.7, 47.6, 48.1, 51.4, 52.1, 56.8, 59.7, 127.5, 129.0, 132.8, 136.0, 163.1. HPLC: 99%. MS m/z (ES+) $[M + H]^+$ = 461.25. HRMS (TOF) m/z calcd for $C_{23}H_{36}N_4SO_3$ $(M + H)^+$, 461.2580; found, 461.2581.

(4-Cyclobutyl-1,4-diazepan-1-yl)(8-(isopropylsulfonyl)-2,8-diazaspiro[4.5]decan-2-yl)methanone (41d). The title compound was made in an analogous manner to **41c** using isopropyl sulfonyl choride to give the title compound in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (d, J = 6.8 Hz, 6H), 1.52−1.95 (m, 12H), 1.95−2.10 (m, 2H), 2.39−2.48 (m, 2H), 2.48−2.59 (m, 2H), 2.77−2.91 (m, 1H),

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

3.11−3.31 (m, 5H), 3.35−3.50 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.4, 16.7, 27.9, 28.2, 34.6, 35.9, 39.6, 43.8, 46.6, 47.6, 48.0, 51.4, 52.1, 53.2, 57.4, 59.6, 163.1. HRMS (TOF) m/z calcd for $C_{21}H_{38}N_4O_3S$ $(M + H)^+$, 427.2737; found, 427.2738.

(8-Benzoyl-2,8-diazaspiro[4.5]decan-2-yl)(4-cyclobutylpiperazin-1-yl)methanone (41e). To *tert*-butyl 2-(4-cyclobutylpiperazine-1-carbonyl)-2,8-diazaspiro[4.5]decane-8-carboxylate **40e** (980 mg, 2.33 mmol) was added 6 N HCl in *i*PrOH (5 mL). The reaction mixture was stirred 4 h, and then Et_2O (50 mL) was added. The salt was filtered under N_2 and dried in vacuo to yield the intermediate amine (800 mg, 91%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 1.71−1.98 (m, 8H), 2.22−2.41 (m, 4H), 2.89−3.03 (m, 2H), 3.12−3.28 (m, 6H), 3.37 (s, 2H), 3.40−3.49 (m, 2H), 3.54 (t, J = 7.1 Hz, 2H), 3.65−3.80 (m, 1H), 3.87−3.97 (m, 2H). This intermediate (200 mg, 0.53 mmol) and Et_3N (294 μL, 2.11 mmol) were mixed in a flask in CH_2Cl_2 (3 mL) and stirred 10 min. Benzoyl chloride (76 mg, 0.53 mmol) was added, and the reaction mixture was stirred for 2 h. The reaction mixture was diluted with CH_2Cl_2 and washed with 5% $NaHCO_3$ and brine and then dried over anhydrous Na_2SO_4. The organic phase was filtered and concentrated in vacuo. The residue was purified via preparatory HPLC using procedure C and then reverse-phase preparatory HPLC (H_2O/ACN, flow: 30 mL/min, 12 g column, 100:0 to 0:100 over 30 min) to give the title compound (190 mg, 88%) as a white solid after lyophilization (H_2O/ACN). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41−1.96 (m, 10H), 1.96−2.10 (m, 2H), 2.25−2.37 (m, 4H), 2.65−2.79 (m, 1H), 3.23−3.51 (m, 10H), 3.54−3.74 (m, 1H), 3.74−3.92 (m, 1H), 7.36−7.44 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 26.9, 34.0, 35.0, 35.6, 39.6, 40.5, 45.2, 45.8, 46.4, 49.2, 57.8, 60.2, 126.8, 128.4, 129.6, 136.0, 162.6, 170.3. MS m/z (ES+) $[M + H]^+$ = 411.31. HRMS (TOF) m/z calcd for $C_{24}H_{34}N_4O_2$ $(M + H)^+$, 411.2754; found, 411.2752.

(4-Cyclobutylpiperazin-1-yl)(8-(phenylsulfonyl)-2,8-diazaspiro[4.5]decan-2-yl)methanone (41f). (4-Cyclobutylpiperazin-1-yl)(2,8-diazaspiro[4.5]decan-2-yl)methanone (200 mg, 0.53 mmol) and Et_3N (220 μL, 1.58 mmol) were mixed in a flask in CH_2Cl_2 (3 mL) and stirred 10 min. Benzenesulfonyl chloride (95 mg, 0.54 mmol) was added, and the reaction mixture was stirred for 2 h. The reaction mixture was diluted with CH_2Cl_2 and washed with 5% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified via preparatory HPLC using procedure C and then reverse-phase preparatory HPLC (H_2O/ACN, flow: 30 mL/min, 12 g column, 100:0 to 0:100 over 30 min) to produce the title compound (200 mg, 85%) as a white solid after lyophilization (H_2O/ACN). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55−1.76 (m, 8H), 1.76−1.93 (m, 2H), 1.93−2.07 (m, 2H), 2.21−2.31 (m, 4H), 2.63−2.75 (m, 1H), 2.77−2.90 (m, 2H), 3.08 (s, 2H), 3.14−3.29 (m, 6H), 3.37 (t, J = 7.1 Hz, 2H), 7.50−7.65 (m, 3H), 7.71−7.79 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 26.9, 33.7, 35.9, 39.3, 43.6, 45.7, 46.4, 49.2, 56.9, 60.2, 127.5, 129.1, 132.8, 136.0, 162.5. Analytical HPLC method D was employed. MS m/z (ES+) $[M + H]^+$ = 447.24.

(8-Benzoyl-2,8-diazaspiro[4.5]decan-2-yl)(1-cyclobutylpiperidin-4-yl)methanone (41g). A mixture of *tert*-butyl 4-(8-benzoyl-2,8-diazaspiro[4.5]decane-2-carbonyl)piperidine-1-carboxylate (40 mg, 0.09 mmol) and a 4 M solution of HCl (0.2 mL, 0.80 mmol) in dioxane was stirred at room temperature for 2 days. The mixture was then concentrated under reduced pressure, basified with a solution of $NaHCO_3$ (sat.), and extracted with EtOAc (3×). The combined organic phases were then dried and concentrated under reduced pressure. The crude material was loaded on a 4 g silica gel column and purified on SiO_2, eluting with 5−60% EtOAc in heptane and then flushed with MeOH to provide (8-benzoyl-2,8-diazaspiro[4.5]decan-2-yl)(piperidin-4-yl)methanone (20 mg, 64.1%) as an oil. The product was analyzed by analytical HPLC MS using procedure D. MS m/z (ES+) $[M + H]^+$ = 356.3. t_R = 1.60 min.

To a solution of (8-benzoyl-2,8-diazaspiro[4.5]decan-2-yl)-(piperidin-4-yl)methanone (15 mg, 0.04 mmol) in DMF (3 mL) was added cyclobutanone (2.96 mg, 0.04 mmol), and the mixture was stirred for 30 min. $NaCNBH_4$ (2.65 mg, 0.04 mmol) was then added, and the mixture was stirred overnight. The mixture was then quenched with a saturated solution of $NaHCO_3$ and concentrated under reduced pressure. The residue was diluted in EtOAc, washed with brine, and concentrated under reduced pressure. The crude material was purified by preparative HPLC using procedure B to provide the title compound (7.0 mg, 37%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 0.86−0.99 (m, 2H), 1.24−1.49 (m, 6H), 1.52−1.64 (m, 1H), 1.64−1.77 (m, 1H), 1.80−2.13 (m, 5H), 2.15−2.28 (m, 1H), 2.28−2.43 (m, 1H), 2.77−2.93 (m, 1H), 3.41−3.62 (m, 5H), 3.62−3.89 (m, 6H), 4.19−4.26 (m, 1H), 7.35−7.44 (m, 2H), 7.44−7.53 (m, 3H). HRMS (TOF) m/z calcd for $C_{25}H_{36}N_3O_2$ $(M + H)^+$, 410.2802; found, 410.2807.

(9-Benzoyl-2,9-diazaspiro[5.5]undecan-2-yl)(4-cyclobutyl-1,4-diazepan-1-yl)methanone (41h). This compound was made in an analogous manner to **41b** with commercially available *tert*-butyl 2,9-diazaspiro[5.5]undecane-9-carboxylate to give the title compound in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21−1.97 (m, 14H), 1.97−2.13 (m, 2H), 2.35−2.67 (m, 4H), 2.76−2.94 (m, 1H), 2.94−3.06 (m, 1H), 3.06−3.24 (m, 3H), 3.32−3.52 (m, 6H), 3.52−3.73 (m, 1H), 3.73−3.94 (m, 1H), 7.35−7.43 (m, 5H). HRMS (TOF) m/z calcd for $C_{26}H_{38}N_4O_2$ $(M + H)^+$, 439.3067; found, 439.3077.

***tert*-Butyl 8-Benzoyl-2,8-diazaspiro[4.5]decane-2-carboxylate (43).** A solution of *tert*-butyl 2,8-diazaspiro[4.5]decane-2-carboxylate·HCl (0.13 g, 0.47 mmol), DIPEA (0.164 mL, 0.94 mmol), and benzoic acid (0.057 g, 0.47 mmol) in 2-methyl tetrahydrofuran (5 mL) was stirred for 1 h at room temperature. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-4-ium (0.125 g, 0.52 mmol) was then added, and the mixture was stirred overnight. The mixture was quenched with water, concentrated under reduced pressure, diluted in EtOAc, washed with $NaHCO_3$ (sat.) and brine, and then concentrated under reduced pressure. The crude material was purified on SiO_2 (40 g), eluting with 5−80% EtOAc in heptane to provide *tert*-butyl 8-benzoyl-2,8-diazaspiro[4.5]decane-2-carboxylate (0.100 g, 61.8%) as an oil. The product was analyzed by analytical HPLC MS using procedure D. MS m/z (ES+) $[M + H]^+$ = 345.3. t_R = 2.23 min.

***tert*-Butyl 4-(8-Benzoyl-2,8-diazaspiro[4.5]decane-2-carbonyl)piperidine-1-carboxylate (44).** *tert*-Butyl 8-benzoyl-2,8-diazaspiro[4.5]decane-2-carboxylate (0.10 g, 0.29 mmol) was solubilized in MeOH (2 mL), and a 4 M solution of HCl (0.508 mL, 2.03 mmol) in dioxane was added. The mixture was then stirred at room temperature for 2 days. The mixture was concentrated under reduced pressure to provide phenyl(2,8-diazaspiro[4.5]decan-8-yl)methanone·HCl (0.120 g, 147%) as an oil. The product was analyzed by analytical HPLC-MS using procedure D. MS m/z (ES+) $[M + H]^+$ = 245.4. t_R = 1.42 min. A mixture of phenyl(2,8-diazaspiro[4.5]decan-8-yl)-methanone·HCl (0.12 g, 0.43 mmol), DIPEA (0.136 mL, 0.78 mmol), and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (0.089 g, 0.39 mmol) in methyl THF (5 mL) was stirred at room temperature for 15 min. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-4-ium (0.094 g, 0.39 mmol) was then added, and the mixture was stirred for 1 h. The solvent was removed under reduced pressure. The mixture was diluted in ethyl acetate, washed with a saturated solution of $NaHCO_3$ and brine, and then concentrated under reduced pressure. The crude material was purified on SiO_2, eluting with 5−60% EtOAc in heptane to provide *tert*-butyl 4-(8-benzoyl-2,8-diazaspiro[4.5]-decane-2-carbonyl)piperidine-1-carboxylate (0.040 g, 22.60%) as an oil. The product was analyzed by analytical HPLC MS using procedure D. MS m/z (ES+) $[M + H]^+$ = 456.3 (major peak: 356.3). t_R = 2.14 min.

GTPγS Assay. Functional antagonism of the human H_3R long form (445 amino acids) was quantified using an agonist-induced [^{35}S]GTPγS scintillation proximity binding assay.[29] Briefly, H_3R-CHO-K1 membranes were incubated for 3 h with test compound, 25 nM *R*-α-methyl histamine, 10 μM GDP, 0.2 nM [^{35}S]GTPγS, and 150 μg of WGA PVT SPA beads in a 100 μL total volume. The amount of [^{35}S]GTPγS binding to the SPA beads was measured on a TopCount (PerkinElmer).

Binding Assay. The affinity of test compounds to recombinant rat and human H_3R was determined using a scintillation proximity binding assay with [^{3}H]-*N*-α-methylhistamine as ligand. Human H_3R-CHO-K1 membranes were incubated for 1.5 h with test compound,

dx.doi.org/10.1021/jm4014828 | J. Med. Chem. XXXX, XXX, XXX−XXX

1.4 nM radioligand, and 1750 μg of WGA PVT SPA beads in a 200 μL total volume. Imetit (10 μM) was used to define nonspecific binding. The amount of radioligand binding to the SPA beads was measured on a TopCount (PerkinElmer). Binding to H_3R in rat brain membranes was performed using the same radioligand but in a filtration format using GF/B filters. Bound radioligand was determined by liquid scintillation counting on a TopCount.

VCD Experimental. For each compound, ∼20 mg of sample was dissolved in 0.5 mL of $CDCl_3$. Each solution was then loaded into a 0.1 mm BaF_2 infrared cell for analysis 4 cm^{-1} resolution using a 5 h, dual source, VCD scan protocol. All analyses were conducted using the BioTools ChiralIR instrument. The instrument incorporated a single photoelastic modulator set for polarization modulation at 37.024 kHz with $\lambda/4$ retardation (optimized for acquisition of the spectral region centered around 1400 cm^{-1}). Lock-in amplification with a 30 μs time constant, a 20 kHz high-pass filter, and a 4 kHz low-pass filter was used. Polarity was verified with α-pinene.

Computational Spectral Simulations. A MonteCarlo molecular mechanics search of low-energy conformers for the (*R*) configuration of the Figure 5 compounds was conducted using MacroModel[35] within the Maestro graphical interface. The 32 lowest-energy conformers identified were used as starting points and minimized using density functional theory (DFT) within Gaussian 03. Optimized structures, harmonic vibrational frequencies/intensities, VCD rotational strengths, and free energies at standard temperature and pressure (STP, including zero-point energies) were determined for each conformer. In these calculations, the B3LYP generalized gradient approximation (GGA) exchange-correlation density functional was used in conjunction with the cc-pVTZ basis set. Simulations of infrared and VCD spectra for each conformer were generated using an in-house written program to fit Lorentzian line shapes (16 cm^{-1} line width) to the computed spectra. In this manner, direct comparisons between simulated and experimental spectra were made.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Secondary pharmacological targets and data for Cpd **6s**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: dean.brown@astrazeneca.com.

Present Address

[‖]AstraZeneca Pharmaceuticals, 35 Gatehouse Drive, Waltham, Massachusetts 02451, United States.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge help from Francis Beaulieu, Thalia Daniels, James Hall, Helene Desfosses, Sandrine Leclerc, Bernard A. Lanoue, Ying Zhou, Ying Liu, Eric Miele, Erika Manyak, Debora Roaquin, Torcan Chemical, and Don Pivonka.

■ DEDICATION

[⊥]We dedicate this manuscript to the memory of Thierry Groblewski, who passed away during the time of this effort. His leadership was critical to the advancement of the project, and he will be greatly missed by his friends and colleagues.

■ ABBREVIATIONS USED

ADHD, attention deficit hyperactivity disoreder; AUC, area under the curve; CDI, carbonyldiimidazole; CSF, cerebrospinal fluid; CNS, central nervous system; DMPK, drug metabolism and pharmacokinetics; DIPEA, diisopropyl ethyl amine; EDCI, 1-ethly-3-(3-dimethylaminopropyl)carbodiimide; HOBT, hydroxy benzotriazole; hERG, *human ether-à-go-go-related gene* encoding for potassium ion channel; HBTU, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate; MDCK, Madin-Darby canine kidney; PD, pharmacodynamic; PK, pharmacokinetic; SAR, structure−activity relationships; SFC, supercritical fluid chromatography; TBAB, tetrabutylammonium bromide; TEMPO, (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl; TFA, trifluoracetic acid; THF, tetrahydrofuran; VCD, vibrational circular dichroism

■ REFERENCES

(1) Arrang, J. M.; Garbarg, M.; Schwartz, J. C. Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature* **1983**, *302*, 832−837.

(2) Brioni, J. D.; Esbenshade, T. A.; Garrison, T. R.; Bitner, S. R.; Cowart, M. D. Discovery of histamine H_3 antagonists for the treatment of cognitive disorders and Alzheimer's disease. *J. Pharmacol. Exp. Ther.* **2011**, *336*, 38−46.

(3) Cowart, M.; Hsieh, G.; Black, L. A.; Zhan, Z.; Gomez, E. J.; Pai, M.; Strakhova, M.; Manelli, A.; Carr, T.; Wetter, J.; Lee, A.; Diaz, G.; Garrison, T.; Brioni, J. D. Pharmacological characterization of A-960656, a histamine H_3 receptor antagonist with efficacy in animal models of osteoarthritis and neuropathic pain. *Eur. J. Pharmacol.* **2012**, *684*, 87−94.

(4) Weisler, R. H.; Pandina, G. J.; Daly, E. J.; Cooper, K.; Gassman-Mayer, C. Randomized clinical study of a histamine H_3 receptor antagonist for the treatment of adults with attention-deficit hyperactivity disorder. *CNS Drugs* **2012**, *26*, 421−434.

(5) Medhurst, S. J.; Collins, S. D.; Billinton, A.; Bingham, S.; Dalziel, R. G.; Brass, A.; Roberts, J. C.; Medhurst, A. D.; Chessell, I. P. Novel histamine H_3 receptor antagonist GSK189254 and GSK334429 are efficacious in surgically-induced and virally-induced rat models of neuropathic pain. *Pain* **2008**, *138*, 61−78.

(6) Karagiannidis, I.; Dehning, S.; Sandor, P.; Tarnok, Z.; Rizzo, R.; Wolanczyk, T.; Madruga-Garrido, M.; Hebebrand, J.; Nöthen, M. M.; Lehmkuhl, G.; Farkas, L.; Nagy, P.; Symanska, U.; Anastasiou, Z.; Stathias, V.; Androutsos, C.; Tsironi, V.; Koumoula, A.; Barta, C.; Zill, P.; Mir, P.; Müller, N.; Barr, C.; Paschou, P. Support of the histaminergic hypothesis in Tourette syndrome: Association of the histamine decarboxylase gene in a large sample of families. *J. Med. Genet.* **2013**, *50*, 760−764.

(7) Esbenshade, T. A.; Browman, K. E.; Bitner, R. S.; Strakhova, M.; Cowart, M. D.; Brioni, J. D. The H_3 receptor: An attractive target for the treatment of cognitive disorders. *Br. J. Pharmacol.* **2008**, *154*, 1166−1181.

(8) Passani, M. B.; Lin, J. S.; Hancock, A.; Crochet, S.; Blandina, P. The histamine H_3 receptor as a novel therapeutic target for cognitive and sleep disorders. *Pharmacol. Sci.* **2004**, *25*, 618−625.

(9) Esbenshade, T. A.; Fox, G. B.; Krueger, K. M.; Miller, T. R.; Kang, C. H.; Denny, L. I.; Witte, D. G.; Yao, B. B.; Pan, L.; Wetter, J.; Marsh, K.; Bennani, Y.; Cowart, M. D.; Sullivan, J. P.; Hancock, A. A. Pharmacological properties of ABT-239 [4-(2{2-[(2R)-2-methylpyrrolidinyl]ethyl}benzofuran-5-yl)benzonitrile]: I. Potent and selective histamine H_3 receptor antagonist with drug-like properties. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 165−175.

(10) Fox, G. B.; Esbenshade, T. A.; Pan, J. B.; Radek, R. J.; Krueger, K. M.; Yao, B. B.; Browman, K. E.; Buckley, M. J.; Ballard, M. E.; Komater, V. A.; Miner, H.; Zhang, M.; Faghih, R.; Rueter, L. E.; Bitner, R.; Drescher, K. U.; Wetter, J.; Marsh, K.; Lemaire, M.; Porsolt, R. D.; Benneni, Y. L.; Sullivan, J. P.; Cowart, M. D.; Decker, M. W.; Hancock, A. A. Pharmacological properties of ABT-239 [4-(2{2-[(2R)-2-

methylpyrrolidinyl]ethyl}benzofuran-5-yl)benzonitrile]: II. Neurophysiological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective H_3 receptor antagonist. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 176−190.

(11) Plancher, J.-M. The histamine H_3 receptor as a therapeutic drug target for metabolic disorders: Status, challenges, and opportunities. *Curr. Top. Med. Chem.* **2011**, *11*, 1430−1446.

(12) Celanire, S.; Wijtmans, M.; Talaga, P.; Leurs, R.; de Esch, I. J. P. Histamine H_3 receptors reach out for the clinic. *Drug Discovery Today* **2005**, *10*, 1613−1627.

(13) For a review, see (a) Hudkins, R. L.; Raddatz, R. Recent advances in drug discovery of histamine H_3 antagonists. *Annu. Rep. Med. Chem.* **2007**, *42*, 49−62. For a recent example, see (b) Zhou, D.; Gross, J. L.; Adedoyin, A. B.; Aschmies, S. B.; Brennan, J.; Bowlby, M.; Di, L.; Kubek, K.; Platt, B. J.; Wang, Z.; Zhang, G.; Brandon, N.; Comery, T. A.; Robichaud, A. J. 2-(Pyrrolidin-1-yl)ethyl-3,4-dihydroisoquinolin-1(2*H*)-one derivatives as potent and selective histamine-3 receptor antagonists. *J. Med. Chem.* **2012**, *55*, 2452−2468. (c) Tao, M.; Aimone, L. D.; Huang, Z.; Mathiasen, J.; Raddatz, R.; Lyons, J.; Hudkins, R. L. Optimization of 5-pyridazin-3-one phenoxypropylamines as potent, selective histamine H_3 receptor antagonists with potent cognition enhancing activity. *J. Med. Chem.* **2012**, *55*, 414−423. (d) For a recent review, see: Singh, M.; Jadhav, H. R. Histamine H_3 receptor function and ligands: Recent developments. *Mini-Rev. Med. Chem.* **2013**, *13*, 47−57. (e) Kuhne, S.; Wijtmans, M.; Lim, H. D.; Leurs, R.; de Esch, I. J. P. Several down, a few to go: Histamine H_3 receptor ligands making the final push towards the market. *Expert Opin. Invest. Drugs* **2011**, *20*, 1629−1648. (f) Allison, B. D.; Carruthers, N. I.; Grice, C. A.; Letavic, M. A. Cyclopropyl amines as modulators of the histamine H_3 receptor. U.S. Patent Appl. 2007/0066821 A1, March 22, 2007.

(14) Hancock, A. A. The challenge of drug discovery of a GPCR target: Analysis of preclinical pharmacology of histamine H_3 antagonists/inverse agonists. *Biochem. Pharmacol.* **2006**, *71*, 1103.

(15) Ritchie, T. J.; MacDonald, S. J. F. The impact of aromatic ring count on compound developability: Are too many aromatic rings a liability in drug design. *Drug Discovery Today* **2009**, *14*, 1011−1020.

(16) hERG pharmacophore: Cavalli, A.; Poluzzi, E.; De Ponti, F.; Recanatini, M. Toward a pharmacophore for drugs inducing the long QT syndrome: Insights from a CoMFA study of HERG K^+ channel blockers. *J. Med. Chem.* **2002**, *45*, 3844−3853.

(17) Swanson, D. M.; Wilson, S. J.; Boggs, J. D.; Xiao, W.; Apodaca, R.; Barbier, A. J.; Lovenberg, T. W.; Carruthers, N. I. Apylsamine-1 and related analogs as histamine H_3 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 897−900.

(18) (a) Santora, V. J.; Covel, J. A.; Hayashi, R.; Hofilena, B. J.; Ibarra, J. B.; Pulley, M. D.; Weinhouse, M. I.; Sengupta, D.; Duffield, J. J.; Semple, G.; Webb, R. R.; Sage, C.; Ren, A.; Pereira, G.; Knudsen, J.; Edwards, J. E.; Suarez, M.; Frazer, J.; Thomsen, W.; Hauser, E.; Whelan, K.; Grottick, A. J. A new family of H_3 receptor antagonists based on the natural product conessine. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1490−1494. (b) Zhao, C.; Sun, M.; Bennani, Y. L.; Gopalakrishnan, S. M.; Witte, D. G.; Miller, T. R.; Krueger, K. M.; Browman, K. E.; Thiffault, C.; Wetter, J.; Marsh, K. C.; Hancock, A. A.; Esbenshade, T. A.; Cowart, M. D. The alkaloid conessine and analogues as potent histamine H_3 receptor antagonists. *J. Med. Chem.* **2008**, *51*, 5423−5430. (c) Lannang, A. M.; Anjum, S.; Tangmouo, J. G.; Krohn, K.; Choudhary, M. I. Conessin isolated from *Holarrhena floribunda*. *Acta Crystallogr.* **2007**, *E63*, o3980.

(19) Barbier, A. J.; Berridge, C.; Laposky, A. D.; Wilson, S. J.; Boggs, J.; Alusio, B.; Mazur, C.; Pudiak, C. M.; Langlois, X.; Xiao, W.; Apodaca, R.; Carruthers, N. I.; Lovenberg, T. W. Acute wake-promoting actions of JNJ-5207852, a novel, diamine-based H_3 antagonist. *Br. J. Pharmacol.* **2004**, *143*, 649−661.

(20) Dvorak, C. A.; Apodaca, R.; Barbier, A. J.; Berridge, C. W.; Wilson, S. J.; Boggs, J. D.; Xiao, W.; Lovenberg, T. W.; Carruthers, N. I. 4-Phenoxypiperidines: Potent, conformationally restricted, non-imidazole histamine H_3 antagonists. *J. Med. Chem.* **2005**, *48*, 2229−2238.

(21) Malmlof, K.; Zaragoza, F.; Golozoubova, V.; Refsgaard, H. H. F.; Cremers, T.; Raun, K.; Wulff, B. S.; Johansen, P. B.; Westerink, B.; Rimvall, K. Influence of a selective histamine receptor antagonist on hypothalamic neural activity, food intake, and body weight. *Int. J. Obes.* **2005**, *29*, 1402−1412.

(22) Medhurst, A. D.; Briggs, M. A.; Bruton, G.; Calver, A. R.; Chessell, I.; Crook, B.; Davis, J. B.; Davis, R. P.; Foley, A. G.; Heslop, T.; Hirst, W. D.; Medhurst, S. J.; Ociepka, S.; Ray, A.; Regan, C. M.; Sargent, B.; Schogger, J.; Stean, T. O.; Trail, B. K.; Upton, N.; White, T.; Orlek, B.; Wilson, D. M. Structurally novel histamine H_3 receptor antagonists GSK207040 and GSK 334429 improve scopolamine-induced memory impairment and capsaicin-induced secondary allodynia in rats. *Biochem. Pharmacol.* **2007**, *73*, 1182−1194.

(23) (a) Letavic, M. A.; Aluisio, L.; Atack, J. R.; Bonaventure, P.; Carruthers, N. I.; Dugovic, C.; Everson, A.; Feinstein, M. A.; Fraser, I. C.; Hoey, K.; Jiang, X.; Keith, J. M.; Koudriakova, T.; Leung, P.; Lord, B.; Lovenberg, T. W.; Ly, K. S.; Morton, K. L.; Motley, S. T.; Nepomuceno, D.; Rizzolio, M.; Rynberg, R.; Sepassi, K.; Shelton, J. Pre-clinical characterization of aryloxypyridine amides as histamine H_3 receptor antagonists: Identification of candidates for clinical development. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4210−4214. (b) Galici, R.; Rezvani, A. H.; Aluisio, L.; Lord, B.; Levin, E. D.; Fraser, I.; Boggs, J.; Welty, N.; Shoblock, J. R.; Motley, S. T.; Letavic, M. A.; Carruthers, N. I.; Dugovic, C.; Lovenberg, T. W.; Bonaventure, P. JNJ-39220675, a novel selective histamine H_3 receptor antagonist, reduces the abuse-related effects of alcohol in rats. *Psychopharmacology* **2011**, *214*, 829−841.

(24) 1-Cyclopropylpiperazine: Gillaspy, M. L.; Lefker, B. A.; Hada, W. A.; Hoover, D. J. A simple method for the formation of cyclopropylamines: The first synthesis of tricyclopropylamine. *Tetrahedron Lett.* **1995**, *36*, 7399−7402. (b) Preparation of 1-cyclopentylpiperazine dihydrochloride (for experimental): Zaragoza, F.; Stephensen, H.; Knudsen, S. M.; Pridal, L.; Wulff, B. S.; Rimvall, K. 1-Alkyl-a-acylpiperazines as a new class of imidazole-free H_3 receptor antagonists. *J. Med. Chem.* **2004**, *47*, 2833−2838.

(25) Wolfe, J. P.; Wagaw, S.; Marcoux, J.-F.; Buchwald, S. Rational development of practical catalysts for aromatic carbon-nitrogen bond formation. *Acc. Chem. Res.* **1998**, *31*, 805−818.

(26) (a) Nafie, L. A.; Dukor, R. K. In *Applications of Vibrational Optical Activity in the Pharmaceutical Industry*; Pivonka, D. E., Chalmers, J. M., Griffiths, P. R., Eds.; John Wiley and Sons: Chichester, West Sussex, England, 2007; pp 129−154. (b) Crawford, T. D. Ab initio calculation of molecular chirooptical properties. *Theor. Chem. Acc.* **2006**, *115*, 227−245. (c) Wesolowski, S. S.; Pivonka, D. E. A rapid alternative to X-ray crystallography for chiral determination: Case studies of vibrational circular dichroism (VCD) to advance drug discovery projects. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4019−4025.

(27) Kaneko, T.; Clark, R. S. J.; Ohi, N.; Ozaki, F.; Kawahara, T.; Kamada, A.; Okano, K.; Yokohama, H.; Ohkuro, M.; Muramoto, K.; Takenaka, O.; Kobayashi, S. Piperidine carboxylic acid derivatives of 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine as orally-active adhesion molecule inhibitors. *Chem. Pharm. Bull.* **2004**, *52*, 675−687.

(28) Wellendorph, P.; Goodman, M. W.; Burstein, E. S.; Nash, N. R.; Brann, M. R.; Weiner, D. M. Molecular cloning and pharmacology of functionally distinct isoforms of the human histamine H_3 receptor. *Neuropharmacology* **2002**, *42*, 929−940.

(29) Ferrer, M.; Kolodin, G.; Zuck, P.; Peltier, R.; Berry, K.; Mandala, S.; Rosen, H.; Ota, H.; Ozaki, S.; Inglese, J.; Strulovici, B. A fully automated [^{35}S]GTPγS scintillation proximity assay for the high-throughput screening of Gi-linked G protein-coupled receptors. *Assay Drug Dev. Technol.* **2003**, *1*, 261−273.

(30) This assay used the Cellomics ArrayScan to quantify the accumulation of phospholipid in the I-13.35 cell line after exposure to cationic amphiphilic drugs known to induce PLD in vitro and in vivo. NBD-PE fluorescent lipid probe (Molecular Probes) was used to measure cellular lipid transport taken up by the cellular lysosomes. Inhibited processing and degradation of phospholipids by test compounds led to an accumulation of NBD-PE in the cells. Ethidium

Homodimer (EthD-2) is a dead-cell stain that was used to determine cell viability.
(31) Bernstein, P.; Ciaccio, P.; Morelli, J. Drug induced phospholipidosis. *Annu. Rep. Med. Chem.* **2011**, *46*, 419−430.
(32) Mesens, N.; Steemans, M.; Hansen, E.; Verheyen, G. R.; Van Goethem, F.; Van Gompel, J. Screening for phospholipidosis induced by central nervous drugs: Comparing the predictivity of an in vitro assay to high throughput in silico assays. *Toxicol. In Vitro* **2010**, *24*, 1417−1425.
(33) Sydserff, S.; Sutton, E. J.; Song, D.; Quirk, M. C.; Maciag, C.; Li, C.; Jonak, G.; Gurley, D.; Gordon, J. C.; Christian, E. P.; Doherty, J. J.; Hudzik, T.; Johnson, E.; Mrzljak, L.; Piser, T.; Smagin, G. N.; Wang, Y.; Dan Widzowski, D.; Smith, J. S. Selective α7 nicotinic receptor activation by AZD0328 enhances cortical dopamine release and improves learning and attentional processes. *Biochem. Pharmacol.* **2009**, *78*, 880−888.
(34) Bernstein, P. R.; Brown, D.; Cacciola, J.; Edwards, P.; Folmer, J.; Groblewski, T.; Sylvester, M.; Wesolowski, S. Spirocyclopropylpiperidinecarboxamide derivatives as histamine H_3 receptor agonists and their preparation, pharmaceutical compositions and use in the treatment of diseases, PCT Int. Appl. WO 2008147314 A1, December 4, 2008.
(35) *MacroModel*, version 10.2; Schrödinger, LLC: New York, 2013.
(36) *ROCS*, version 3.1.2; OpenEye Scientific Software, Inc.: Santa Fe, NM, 2011.

dx.doi.org/10.1021/jm4014828 | *J. Med. Chem.* XXXX, XXX, XXX−XXX